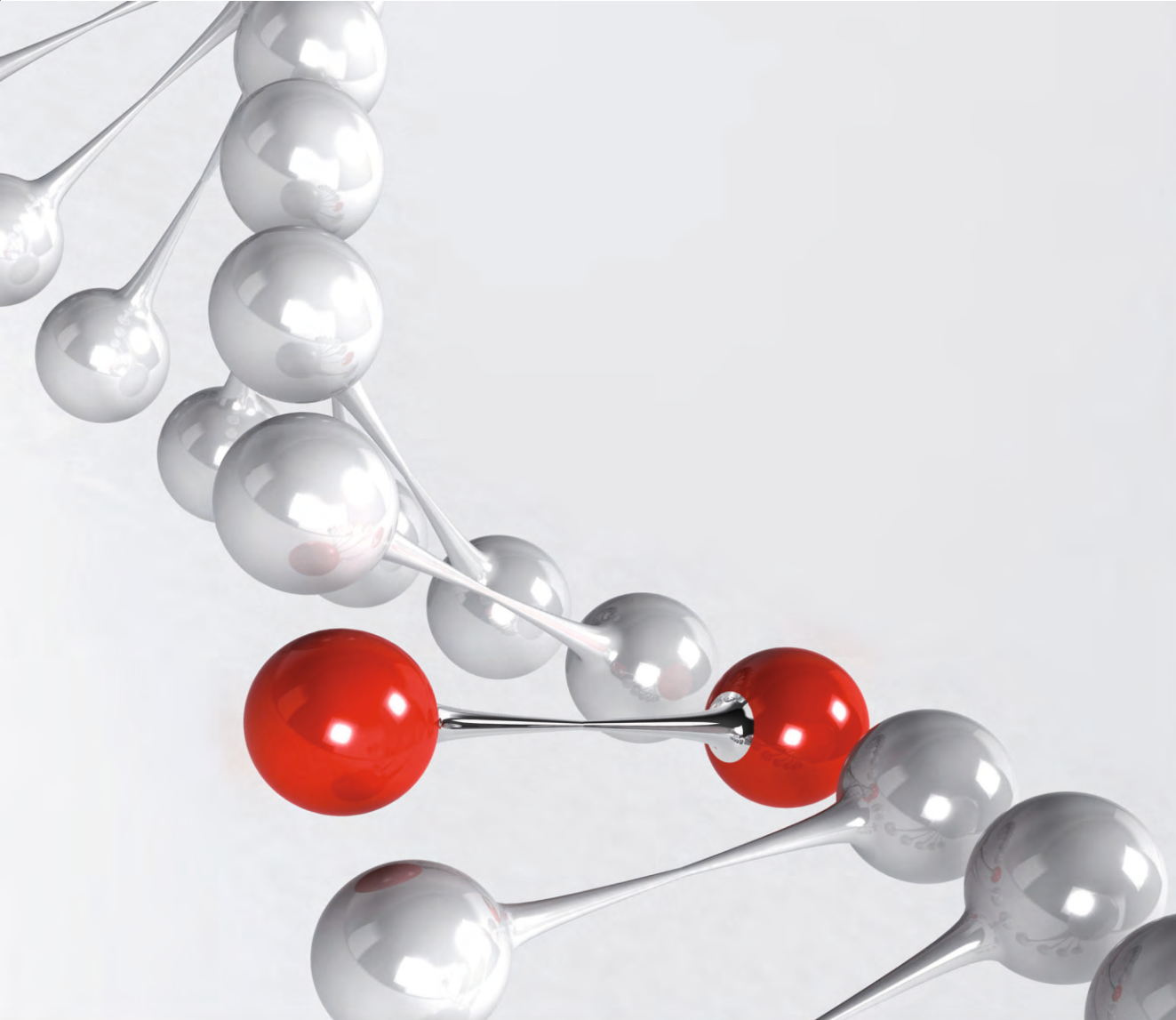




## User manual

# InviMag<sup>®</sup> Plant DNA Mini Kit/ KF96

for use on KingFisher<sup>®</sup> 96 and KingFisher<sup>®</sup> Flex, Thermo Fisher

for automated purification of genomic DNA from up to 100 mg plant material and food of vegetable origin with magnetic beads

REF

7437300Xo



STRATEC Molecular GmbH, D-13125 Berlin

## Instruction for InviMag<sup>®</sup> Plant DNA Mini Kit/ KF96

The **InviMag<sup>®</sup> Plant DNA Mini Kit/ KF96** is the ideal tool for isolation and purification of DNA from up to 100 mg plant material or food from plant origin with the patented InviMag<sup>®</sup> technology using a KF96 / KFflex96 instrument.

The kit is neither validated for the isolation of genomic DNA from cultured or isolated cells, from blood, stool samples, swabs, dried blood stains or cell free body fluids, like cerebrospinal fluid, synovial fluid and urine nor from bacteria, fungi, parasites or purification of RNA.

Trademarks: InviMag<sup>®</sup>, Invisorb<sup>®</sup>. Registered marks, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

The Invisorb<sup>®</sup> technology is covered by patents and patent applications: US 6,110,363, US 6,043,354, US 6,037,465, EP 0880535, WO 9728171, WO 9534569, EP 0765335, DE 19506887, DE 10041825.2, WO 0034463.

InviMag<sup>®</sup> and Invisorb<sup>®</sup> are registered trademarks of STRATEC Biomedical AG.

The PCR process is covered by US Patents 4,683,195, and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG.

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## Kit contents of InviMag® Plant DNA Mini Kit/ KF96

Store the **SNAP Solution** at 2-8°C.

Store diluted **Proteinase K** at -20°C.

Store all other kit components at room temperature!

|                                      | 96 extractions                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | 5 x 96 extractions                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
|--------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Catalogue No.</b>                 | 7437300100                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | 7437300200                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| <b>Lysis Buffer P</b>                | 50 ml                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | 210 ml                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| <b>Proteinase K working solution</b> | 2 x 1.5 ml                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | 10.5 ml                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| <b>Binding Buffer A</b>              | 9 ml<br>(final volume 30 ml)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | 36 ml<br>(final volume 120 ml)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| <b>SNAP Solution</b>                 | 2 x 1.1 ml                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | 10.5 ml                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| <b>Wash Buffer I</b>                 | 80 ml<br>(final volume 160 ml)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | 3 x 80 ml<br>(final volume 3 x 160 ml)                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| <b>Wash Buffer II</b>                | 60 ml<br>(final volume 200 ml)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | 4 x 60 ml<br>(final volume 4 x 200 ml)                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| <b>Elution Buffer</b>                | 15 ml                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | 60 ml                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| <b>2.0 ml Deep Well Plate</b>        | 4 pieces                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | 20 pieces                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| <b>KF96 Tip Comb for DW magnets</b>  | 1 piece                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | 5 pieces                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| <b>200 µl Elution Plate*</b>         | 2 pieces                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | 10 pieces                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| <b>Prefilter Plate I</b>             | 1 piece                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | 5 pieces                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| <b>Sealing Foils</b>                 | 2                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | 10                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| <b>Manual</b>                        | 1                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | 1                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| <b>Initial steps</b>                 | <p>Add 1.5 ml distilled water to each <b>Proteinase K</b> tube, mix thoroughly until completely dissolving and store at -20°C!</p> <p>Add 80 ml of 96-100% ethanol to the bottle <b>Wash Buffer I</b></p> <p>Add 140 ml of 96-100% ethanol to the bottle <b>Wash Buffer II</b>, mix thoroughly and always keep the bottles firmly closed</p> <p>Add 21 ml 99.7% Isopropanol to the <b>Binding Buffer A</b>. Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times.</p> | <p>Dilute <b>Proteinase K</b> in 10.5 ml of ddH<sub>2</sub>O, mix thoroughly until completely dissolving and store at -20°C!</p> <p>Add 80 ml of 96-100% ethanol to each bottle <b>Wash Buffer I</b></p> <p>Add 140 ml of 96-100% ethanol to each bottle <b>Wash Buffer II</b>, mix thoroughly and always keep the bottles firmly closed</p> <p>Add 84 ml 99.7% Isopropanol to the <b>Binding Buffer A</b>. Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times.</p> |

\* Elution and Tip Plate are identically. Use one provided Elution Plate as a Tip Plate.

## Kit contents of InviMag® Plant DNA Mini Kit/ KF96 w/o plastic

Store the **SNAP Solution** at 2-8°C.

Store diluted **Proteinase K** at -20°C.

Store all other kit components at room temperature!

|                                                               | 96 extractions                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | 5 x 96 extractions                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
|---------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Catalogue No.</b>                                          | 7437300150                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | 7437300250                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| <b>Lysis Buffer P</b>                                         | 50 ml                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | 210 ml                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| <b>Proteinase K working solution</b>                          | 2 x 1.5 ml                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | 10.5 ml                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| <b>Binding Buffer A</b>                                       | 9 ml<br>(final volume 30 ml)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | 36 ml<br>(final volume 120 ml)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| <b>SNAP Solution</b>                                          | 2x 1.1 ml                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | 10.5 ml                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| <b>Wash Buffer I</b>                                          | 80 ml<br>(final volume 160 ml)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | 3 x 80 ml<br>(final volume 3 x 160 ml)                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| <b>Wash Buffer II</b>                                         | 60 ml<br>(final volume 200 ml)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | 4 x 60 ml<br>(final volume 4 x 200 ml)                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| <b>Elution Buffer</b>                                         | 15 ml                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | 60 ml                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| <b>Sealing Foils</b>                                          | 2                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | 10                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| <b>Manual</b>                                                 | 1                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | 1                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| <b>Prefilter Plate I</b>                                      | 1                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | 5                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| <b>Initial steps</b>                                          | <p>Add 1.5 ml distilled water to each <b>Proteinase K</b> tube, mix thoroughly until completely dissolving and store at -20°C!</p> <p>Add 80 ml of 96-100% ethanol to the bottle <b>Wash Buffer I</b></p> <p>Add 140 ml of 96-100% ethanol to the bottle <b>Wash Buffer II</b>, mix thoroughly and always keep the bottles firmly closed</p> <p>Add 21 ml 99.7% Isopropanol to the <b>Binding Buffer A</b>. Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times.</p> | <p>Dilute <b>Proteinase K</b> in 10.5 ml of ddH<sub>2</sub>O, mix thoroughly until completely dissolving and store at -20°C!</p> <p>Add 80 ml of 96-100% ethanol to each bottle <b>Wash Buffer I</b></p> <p>Add 140 ml of 96-100% ethanol to each bottle <b>Wash Buffer II</b>, mix thoroughly and always keep the bottles firmly closed</p> <p>Add 84 ml 99.7% Isopropanol to the <b>Binding Buffer A</b>. Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times.</p> |
| <b>Plastic to be supplied by user</b> (see order information) |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| <b>2.0 ml Deep Well Plate</b>                                 | 4                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | 20                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| <b>KF 96 Tip Comb for DW magnets</b>                          | 1                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | 5                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| <b>200 µl Elution Plate*</b>                                  | 2                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | 10                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |

\* Elution and Tip Plate are identically. Use one provided Elution Plate as a Tip Plate.

## Symbols



Manufacturer



Lot number



Catalogue number



Expiry date



Consult operating instructions



Temperature limitation



Do not reuse

## Storage

All buffers and kit components of the InviMag<sup>®</sup> Plant DNA Mini Kit/ KF96, except dissolved Proteinase K and SNAP Solution, should be stored at room temperature and are stable for at least 12 months at these conditions.

**Proteinase K:** Dissolved Proteinase K must be stored at -20°C. We recommended preparing aliquots in order to avoid multiple freezing and thawing cycles because this can lead to decreased enzymatic activity.

**SNAP Solution:** The magnetic particles should be stored at 2-8°C.

**Wash Buffer I, Wash Buffer II, Binding Buffer A:** Buffers charged with either ethanol or isopropanol should be stored at room temperature and must be sealed accordingly. If any precipitates are visible within the provided solutions solve them by carefully warming up to 30°C.

**Room temperature (RT) is defined as range from 15-30°C.**

## Quality control and product warranty

STRATEC Molecular warrants the correct function of the InviMag<sup>®</sup> Plant DNA Mini Kit/ KF96 for applications as described in this manual. Purchaser must determine the suitability of the product for its particular use. Should any product fail to perform the applications as described in the manual, STRATEC Molecular will check the lot and if STRATEC Molecular investigates a problem in the lot, STRATEC Molecular will replace the product free of charge.

STRATEC Molecular reserves the right to change, alter, or modify any product to enhance its performance and design at any time.

In accordance with STRATEC Molecular's ISO 9001-2000 and ISO EN 13485 certified Quality Management System the performance of all components of the InviMag<sup>®</sup> Plant DNA Mini Kit/ KF96 have been tested separately against predetermined specifications routinely on lot-to-lot to ensure consistent product quality.

If you have any questions or problems regarding any aspects of InviMag<sup>®</sup> Plant DNA Mini Kit/ KF96 or other STRATEC Molecular products, please do not hesitate to contact us. A copy of STRATEC Molecular's terms and conditions can be obtained upon request or are presented at the STRATEC Molecular webpage.

**For technical support or further information please contact:**

**from Germany** +49-(0)30-9489-2901/ 2910

**from abroad** +49-(0)30-9489-2907

**or contact your local distributor.**

## Intended use

The **InviMag® Plant DNA Mini Kit/ KF96** is designed for a fully automated preparation of genomic DNA from up to 100 mg plant or food (plant origin) material using the patented **InviMag® technology** in combination with a KF96/ KFflex96 instrument.

The whole process is based on a patented bead technology, used for isolation of genomic DNA by binding the nucleic acid onto magnetic particles in absence of chaotropic buffer components.

For reproducible and high yields appropriate sample storage is essential.

**THE PRODUCT IS INTENDED FOR USE BY PROFESSIONALS ONLY, SUCH AS TECHNICIANS, PHYSICIANS AND BIOLOGISTS TRAINED IN MOLECULAR BIOLOGICAL TECHNIQUES.** It is designed to be used with any downstream application employing enzymatic amplification or other enzymatic modifications of RNA followed by signal detection or amplification. Any diagnostic results generated by using the sample preparation procedure in conjunction with any downstream diagnostic assay should be interpreted with regard to other clinical or laboratory findings.

To minimize irregularities in your results, adequate controls for downstream applications should be used.

## Product use limitation

The kit is neither suitable for the isolation of DNA from blood, serum or plasma, bacteria, fungi or viruses, nor for isolation and purification of RNA.

The included chemicals are only useable once.

Differing of starting material or flow trace may lead to inoperability; therefore neither a warranty nor guarantee in this case will be given, neither implied nor express.

The user is responsible to validate the performance of the STRATEC Molecular product for any particular use. STRATEC Molecular does not provide for validation of performance characteristics of the product with respect to specific applications. STRATEC Molecular products may be used e.g. in clinical diagnostic laboratory systems conditioned upon the complete diagnostic system of the laboratory the laboratory has been validated pursuant to CLIA' 88 regulations in the U.S. or equivalents in other countries.

All products sold by STRATEC Molecular are subject to extensive quality control procedures (according to ISO 9001-2000 and ISO EN 13485) and are warranted to perform as described herein. Any problems, incidents or defects shall be reported to STRATEC Molecular immediately upon detection thereof.

The chemicals and the plastic parts are for laboratory use only; they must be stored in the laboratory and must not be used for purposes other than intended.

The product with its contents is unfit for consumption.

## Safety information

When and while working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles!

Avoid skin contact! Adhere to the legal requirements for working with biological material!

For more information, please consult the appropriate material safety data sheets (MSDS). These are available online in convenient and compact PDF format at [www.stratec.com](http://www.stratec.com) for each STRATEC Molecular product and its components. If buffer bottles are damaged or leaking, **WEAR GLOVES, AND PROTECTIVE GOGGLES** when discarding the bottles in order to avoid any injuries.

STRATEC Molecular has not tested the liquid waste generated by the **InviMag® Plant DNA Mini Kit/ KF96** procedures for residual infectious materials. Contamination of the liquid waste with residual infectious materials is highly unlikely, but cannot be excluded completely. Therefore, the liquid waste has to be considered infectious and be handled and discarded accordingly to local safety regulations.

European Community risk and safety phrases for the components of the **InviMag® Plant DNA Mini Kit/ KF96** to which they apply, are listed below as follows:

### Lysis Buffer P



danger  
H319 P305-351-338

### Proteinase K:



danger  
H315-319-334-335 P280-305-351-338-310-405

### Wash Buffer I



warning  
H302-312-332-412 EUH032 P273

|                        |                                                                                                                  |
|------------------------|------------------------------------------------------------------------------------------------------------------|
| <b>H315:</b>           | Causes skin irritation.                                                                                          |
| <b>H319:</b>           | Causes serious eye irritation.                                                                                   |
| <b>H334:</b>           | May cause allergy or asthma symptoms or breathing difficulties if inhaled.                                       |
| <b>H335:</b>           | May cause respiratory irritation.                                                                                |
| <b>H302:</b>           | Harmful if swallowed.                                                                                            |
| <b>H312:</b>           | Harmful in contact with skin.                                                                                    |
| <b>H332:</b>           | Harmful if inhaled.                                                                                              |
| <b>H412:</b>           | Harmful to aquatic life with long lasting effects.                                                               |
| <b>EUH032:</b>         | Contact with acids liberates very toxic gas.                                                                     |
| <b>P280:</b>           | Wear protective gloves/protective clothing/eye protection/face protection.                                       |
| <b>P305+P351+P338:</b> | If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses if present. Continue rinsing. |
| <b>P310:</b>           | Immediately call a POISON CENTER or doctor/physician.                                                            |
| <b>P405:</b>           | Store locked up.                                                                                                 |
| <b>P273:</b>           | Avoid release to the environment.                                                                                |

**Emergency medical information can be obtained 24 hours a day from infotrac:**

**outside of USA:** 1 – 352 – 323 – 3500  
**inside of USA :** 1 – 800 – 535 – 5053

## Product characteristic of the InviMag® Plant DNA Mini Kit/ KF96

| Starting material           | Yield                                                          | Time                         | Ratio                        |
|-----------------------------|----------------------------------------------------------------|------------------------------|------------------------------|
| up to 100 mg plant material | 5 - 25 µg; depends on the kind and amount of starting material | about 30 min (without lysis) | $A_{260}:A_{280}$<br>1.8-2.2 |

The **InviMag® Plant DNA Mini Kit/ KF96** is designed for a semi-automated preparation of genomic DNA from up to 100 mg of plant material using magnetic beads and the KF96 or KFflex96 workstation. The isolation process is based on the patented **InviMag®** technology.

The DNA isolation process relies on the interaction of nucleic acids with coated magnetic particles at adapted buffer conditions. The KF96 / KFflex96 instrument performs all purification steps of the DNA purification procedure automatically, except the plant sample preparation, lysis and initial loading of the system. Sample cross-contamination and reagent cross-over is effectively eliminated by the provided assay file.

The KingFisher® instrument uses magnetic rods to transport the DNA bound to magnetic particles through the various assay phases like binding, washing, drying and elution. The volume of buffers and other liquids required for DNA isolation is reduced to a minimum.

To achieve optimal lysis conditions and high yields, the plant samples are first mechanically disrupted followed by a lysis step in an optimized buffer system at elevated temperature. After lysis, a binding step is performed in which the DNA is bound to the magnetic particles followed by several washing steps before the pure DNA is finally eluted.

The purified and high quality DNA can be stored at -20°C for subsequent use and is ready-to-use for subsequent downstream applications like:

- PCR\*
- Genotyping
- Restriction digestion

No toxic or hazardous chemicals are used.

For the isolation of DNA from single plant samples STRATEC Molecular offers the **Invisorb® Spin Plant Mini Kit**, as well as 96 well kits for use in a centrifuge.

For further information please contact +49 (0) 30 9489 2901 or 2910 in Germany and +49 (0) 30 9489 2907 from foreign countries or ask your local distributor.

## Sampling and storage of starting material

Harvested plant samples can be stored at room temperature for up to 2–3 hours. For short-term storage (up to one week) samples may be stored at 2–8°C. For long-term storage, we recommend freezing samples at -20°C or -80°C. Multiple thawing and freezing cycles before isolating the DNA should be avoided because this can lead to degraded DNA and reduced yields.

STRATEC Molecular will be released of its responsibilities if other sample materials than described in the Intended Use are processed or if the sample preparation protocols are changed or modified.

## Principle and Procedure

### Lysis

Samples are disrupted by using a mixer mill, bead mill or by mortar and pestle in combination with liquid nitrogen. Afterwards, the disrupted material is lysed at denaturing non-chaotropic conditions at elevated temperatures in presence of **Lysis Buffer P** and **Proteinase K**. Because unlysed material has to be removed after lysis by filtration, the lysis procedure has to be performed externally. The filtration step is required, because in most cases a centrifugation step will not be able to remove very small debris, especially if a mortar and pestle was used for disrupting.

### Binding of the DNA

After addition of **Binding Buffer A** and **SNAP Solution** to the lysate, optimal binding conditions are adjusted and the genomic DNA is bound to the magnetic particles.

### Removing residual contaminants

Contaminants are efficiently removed using **Wash Buffer I** and **Wash Buffer II**, while the DNA remains bound to the magnetic beads.

### Elution

The DNA is finally eluted in **Elution Buffer**. The eluted DNA is ready-to-use in different subsequent downstream applications like:

- o PCR\*, RAPD, AFLP analysis
- o microsatellite analysis
- o genotyping
- o enzymatic restriction digestion

## Yield and quality of genomic DNA

The amount of purified DNA derived by the **InviMag® Plant DNA Mini Kit /KF96** procedure from plant materials depends on the sample source, transport conditions, storage and sample age.

The overall yield and quality of the isolated genomic DNA is suitable for any detection system.

\*The PCR process is covered by US Patents 4,683,195, and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG.

## Important points before starting a protocol

Immediately upon receipt of the product, inspect the product and its components as well as the package for any apparent damages, correct quantities and quality. If there are any unconformities you have to notify STRATEC Molecular in writing with immediate effect upon inspection thereof. If buffer bottles are damaged, contact the STRATEC Molecular Technical Services or your local distributor. In case of liquid spillage, refer to "Safety Information" (see page 7). Do not use damaged kit components, since their use may lead to poor kit performance.

- Change pipet tips between liquid transfers. To avoid cross-contamination, we recommend the use of aerosol-barrier pipet tips.
- When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles.
- Discard contaminated gloves immediately.
- Do not combine components of different kits, unless the lot numbers are identical.
- Avoid microbial contamination of the kit reagents.
- This kit should only be used by trained personnel.

## Preparing reagents and buffers

Before starting a run, bring all reagents to room temperature. Gently mix and redissolve any precipitates by warming up to 30°C. Swirl gently to avoid foaming.

**Lysis Buffer P** and **Elution Buffer** are ready-to-use.

Add the required amount of ddH<sub>2</sub>O to the reaction tube containing the **Proteinase K**. Vortex for 5 s and keep the solution on ice. Store unused and diluted **Proteinase K** at –20°C.

| 1x 96 DNA-extractions                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Add 21 ml 99.7% Isopropanol to the <b>Binding Buffer A</b> . Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times.<br>Dilute <b>Proteinase K</b> by addition of 1.5 ml of ddH <sub>2</sub> O, mix thoroughly until completely dissolving and store at -20°C!<br>Add 80 ml of 96-100% ethanol to the bottle <b>Wash Buffer I</b><br>Add 140 ml of 96-100% ethanol to the bottle <b>Wash Buffer II</b> .<br>Mix thoroughly and always keep the bottle firmly closed |
| 5x 96 DNA-extractions                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| Add 84 ml 99.7% Isopropanol to the <b>Binding Buffer A</b> . Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times.<br>Dilute <b>Proteinase K</b> by addition of 10.5 ml of ddH <sub>2</sub> O, mix thoroughly until completely dissolving and store at -20°C!<br>Add 80 ml of 96-100% ethanol to the bottle <b>Wash Buffer I</b><br>Add 140ml of 96-100% ethanol to the bottle <b>Wash Buffer II</b><br>Mix thoroughly and always keep the bottle firmly closed   |

## Reagents and equipment to be supplied by user

- Measuring cylinder (250 ml)
- Pipette and pipette tips
- Disposable gloves
- ddH<sub>2</sub>O
- Vortexer
- 96-100% ethanol
- Isopropanol

\*The **InviMag® Plant DNA Mini Kit/ KF96** is validated with 2-Propanol; Rotipuran >99.7%, p.a., ACS, ISO (Order no. 6752) from **Carl Roth**

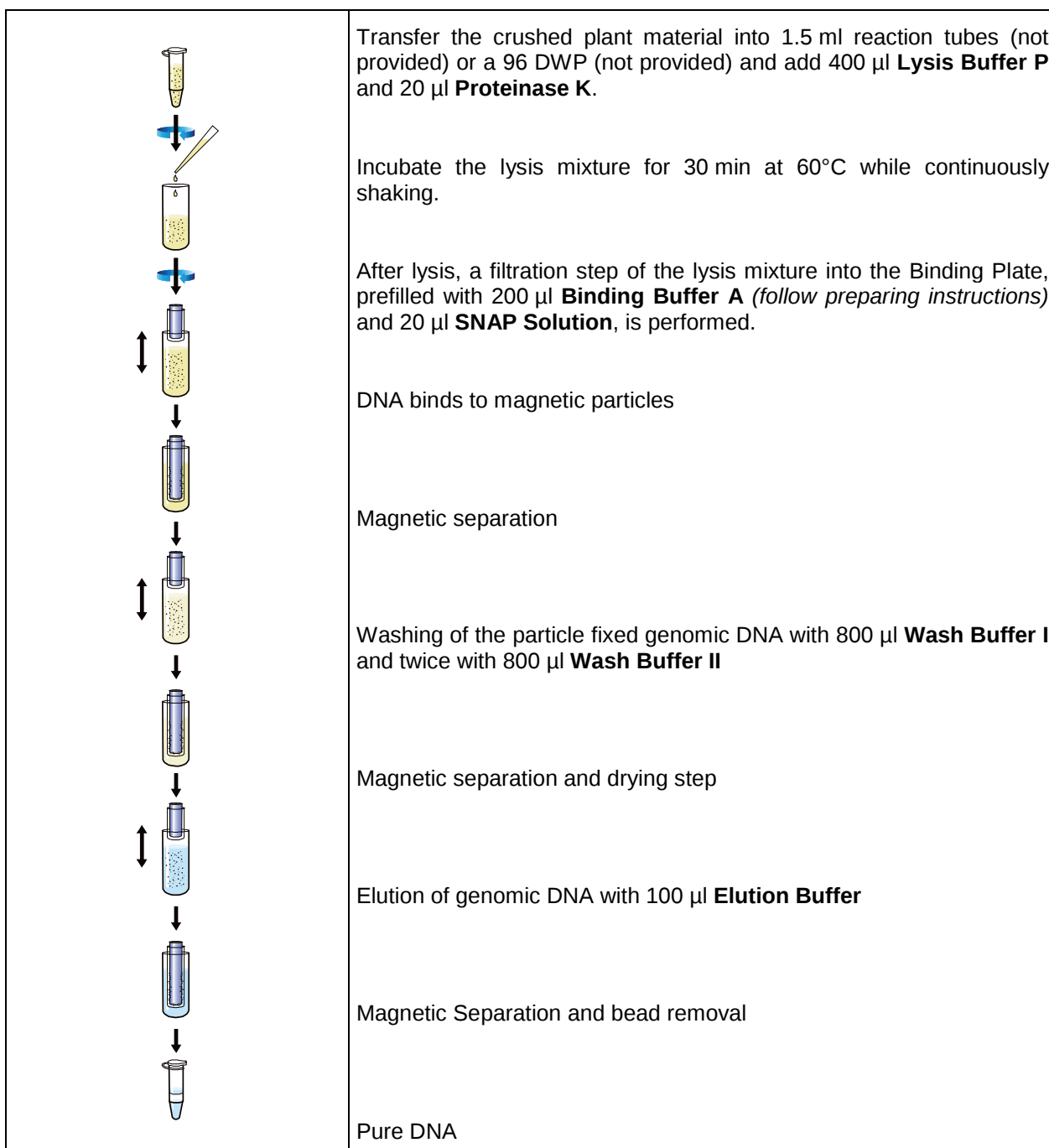
\* Possible suppliers for Isopropanol:

**Carl Roth**  
2-Propanol  
Rotipuran >99.7%, p.a., ACS, ISO  
Order no. 6752

**Applichem**  
2-Propanol für die Molekularbiologie  
Order Nr. A3928

**Sigma**  
2-Propanol  
Order no. 59304-1L-F

## Scheme of the InviMag® Plant DNA Mini Kit/ KF96



## Lysis Procedures

### Protocol: Isolation of genomic DNA from up to 100 mg of plant material

**Please read the instructions carefully and conduct the prepared procedure.**

---

#### Homogenization of the starting material

Homogenize up to 100 mg of plant material by use of a pestle and mortar in combination with liquid nitrogen. Commercially available equipment for homogenization (bead mill, etc.) can be used too.

Transfer the homogenized starting material either into 1.5 ml reaction tubes (not provided) or use a 96 DWP (not provided) and add 400 µl of Lysis Buffer P and 20 µl of Proteinase K to each sample. Incubate the mixtures at 60°C for 30 min while continuously shaking.

During lysis, prefill all plates with the required buffers and appropriate volumes (see "Starting a run", page 12). After lysis, set up the Prefilter plate I onto the Binding Plate (2.0 ml Deep Well Plate) and transfer the lysis mixtures into the filter plate. Incubate for 2-3 min at room temperature or until all lysates have passed the filter plate. The lysed samples have now been transferred into the Binding Plate.

Remove the filter plate and start the run on the KF instrument (see below).

**Important:** The kit will also co-purify RNA beside DNA. For the elimination of RNA (if required) add 20 µl RNase A (10 mg/ml) to the Lysis Buffer P prior before starting the lysis procedure.

### Starting a Run on a KF96 / KFflex96 instrument

**Attention:** Please be aware, that you have to prepare the **Binding Buffer A** – see instruction page: 10

**Note:** Before starting the purification process with the KingFisher instrument please carefully read the manufacturer's manual! Resuspend/Vortex the magnetic particles (SNAP Solution) thoroughly before use!

1. Switch on the KingFisher instrument
2. Prefill all required plates as described below:

**Tip Plate:** Place the KF96 Tip Comb for DW magnets on a Tip Plate (Use one provided Elution Plate as Tip Plate. These are identical.)

**Binding Plate:** 200 µl **Binding Buffer A**, 20 µl **SNAP Solution** to a 2 ml Deep Well Plate

**Washing\_Plate\_1:** Add 800 µl **Wash Buffer I** to a 2 ml Deep Well Plate

**Washing\_Plate\_2:** Add 800 µl **Wash Buffer II** to a 2 ml Deep Well Plate

**Washing\_Plate\_3:** Add 800 µl **Wash Buffer II** to a 2 ml Deep Well Plate

**Elution Plate:** Add 100 µl **Elution Buffer** to the 200 µl Elution Plate

3. Choose either the assay file "**InviMag\_Plant\_KF96**" (KF96 assay file) or "**InviMag\_Plant\_KFflex96**" (KFflex96 assay file) on the display of the corresponding KingFisher instrument and press the "START" button.
4. Insert the prefilled plates onto the right positions of the KingFisher instrument by following the specifications shown on the instrument display and confirm every loading step with the "START" button. When all prefilled plates are loaded press the "START" button to initialize the assay file. The assay file will start at the Binding Step. From this point, the instrument will continue with the purification process without any further user interaction.

## **The following extraction steps run automatically on the KingFisher System!**

### **1. Binding of the DNA**

Automatically sample mixing for 5 min. SNAPs separation. Transfer of the SNAPs to Washing Plate 1.

### **2. First Washing**

Automatically sample mixing for 1.5 min. SNAPs separation. Transfer of the SNAPs to Washing Plate 2.

### **3. Second Washing**

Automatically sample mixing for 1 min. SNAPs separation. Transfer of the SNAPs to Washing Plate 3.

### **4. Third Washing**

Automatically sample mixing for 1 min. SNAPs separation.

### **5. Drying**

Drying of SNAPs outside Washing Plate 3 for 5 minutes. Transfer of the SNAPs to the Elution Plate.

### **6. Elution of the DNA**

Incubation of SNAPs for 10 minutes at 65°C while continuously mixing. SNAPs separation. Removal of SNAPs into Washing Plate 3 (disposal).

### **Important Notes:**

1. *After finishing the extraction protocol, the Elution Plate contains the extracted DNA. Store the DNA at adequate conditions. For long-term storage we recommend to store the DNA at -20°C.*
2. *If the extracted DNA contains carryover from magnetic particles, transfer the DNA into a new 1.5 ml reaction tube and centrifuge at maximum speed for 1 minute. Transfer the clear supernatant (containing the DNA) into a new tube.*

The eluted DNA is ready-to-use in different downstream applications. The eluted DNA can be stored for several weeks at 4-8°C or stored at -20°C for long-term storage.

## For self-programming of the KF96 / KFlex96 instrument

### Reagent info

| Tip Plate       |                                          | KingFisher 96 KF plate |                           |         |
|-----------------|------------------------------------------|------------------------|---------------------------|---------|
|                 | Name                                     | Well volume [µl]       | Total reagent volume [µl] | Type    |
|                 | -                                        | -                      | -                         | -       |
| Binding Plate   |                                          | Microtiter DW 96 plate |                           |         |
|                 | Name                                     | Well volume [µl]       | Total reagent volume [µl] | Type    |
|                 | Crushed sample material in LysisBuffer P | 420                    | -                         | Sample  |
|                 | Binding Buffer A                         | 200                    | -                         | Reagent |
|                 | SNAP Solution                            | 20                     | -                         | Reagent |
| Washing Plate 1 |                                          | Microtiter DW 96 plate |                           |         |
|                 | Name                                     | Well volume [µl]       | Total reagent volume [µl] | Type    |
|                 | Wash Buffer I                            | 800                    | -                         | Reagent |
| Washing Plate 2 |                                          | Microtiter DW 96 plate |                           |         |
|                 | Name                                     | Well volume [µl]       | Total reagent volume [µl] | Type    |
|                 | Wash Buffer II                           | 800                    | -                         | Reagent |
| Washing Plate 3 |                                          | Microtiter DW 96 plate |                           |         |
|                 | Name                                     | Well volume [µl]       | Total reagent volume [µl] | Type    |
|                 | Wash Buffer II                           | 800                    | -                         | Reagent |
| Elution Plate   |                                          | KingFisher 96 KF plate |                           |         |
|                 | Name                                     | Well volume [µl]       | Total reagent volume [µl] | Type    |
|                 | Elution Buffer                           | 100                    | -                         | Reagent |

### Dispensed reagents

The protocol does not contain dispensed reagents

## Steps data

|                                                                                     |                   |                          |                     |
|-------------------------------------------------------------------------------------|-------------------|--------------------------|---------------------|
|    | Tip 1             | 96 DW tip comb           |                     |
|    | Pick-Up           | Tip Plate                |                     |
|    | Binding Step      | Binding Plate            |                     |
|                                                                                     | Beginning of step | Precollect               | No                  |
|                                                                                     |                   | Release time, speed      | 00:00:15, Fast      |
|                                                                                     | Mixing / heating: | Mixing time, speed       | 00:05:00, Medium    |
|                                                                                     |                   | Heating during mixing    | No                  |
|                                                                                     | End of step       | Postmix                  | No                  |
|                                                                                     |                   | Collect count            | 3                   |
|                                                                                     |                   | Collect time [s]         | 10                  |
|    | Washing Step 1    | Washing Plate 1          |                     |
|                                                                                     | Beginning of step | Precollect               | No                  |
|                                                                                     |                   | Release time, speed      | 00:00:10, Fast      |
|                                                                                     | Mixing / heating: | Mixing time, speed       | 00:01:30, Fast      |
|                                                                                     |                   | Heating during mixing    | No                  |
|                                                                                     | End of step       | Postmix                  | No                  |
|                                                                                     |                   | Collect count            | 3                   |
|                                                                                     |                   | Collect time [s]         | 5                   |
|    | Washing Step 2    | Washing Plate 2          |                     |
|                                                                                     | Beginning of step | Precollect               | No                  |
|                                                                                     |                   | Release time, speed      | 00:00:10, Fast      |
|                                                                                     | Mixing / heating: | Mixing time, speed       | 00:01:00, Fast      |
|                                                                                     |                   | Heating during mixing    | No                  |
|                                                                                     | End of step       | Postmix                  | No                  |
|                                                                                     |                   | Collect count            | 3                   |
|                                                                                     |                   | Collect time [s]         | 5                   |
|  | Washing Step 3    | Washing Plate 3          |                     |
|                                                                                     | Beginning of step | Precollect               | No                  |
|                                                                                     |                   | Release time, speed      | 00:00:10, Fast      |
|                                                                                     | Mixing / heating: | Mixing time, speed       | 00:01:00, Fast      |
|                                                                                     |                   | Heating during mixing    | No                  |
|                                                                                     | End of step       | Postmix                  | No                  |
|                                                                                     |                   | Collect count            | 3                   |
|                                                                                     |                   | Collect time [s]         | 5                   |
|  | Drying            | Washing Plate 3          |                     |
|                                                                                     |                   | Dry time                 | 00:05:00            |
|                                                                                     |                   | Tip position             | Outside well / tube |
|  | Elution           | Elution Plate            |                     |
|                                                                                     | Beginning of step | Precollect               | No                  |
|                                                                                     |                   | Release time, speed      | 00:00:10, Medium    |
|                                                                                     | Mixing / heating: | Mixing time, speed       | 00:10:00, Slow      |
|                                                                                     |                   | Heating temperature [°C] | 65                  |
|                                                                                     |                   | Preheat                  | Yes                 |
|                                                                                     | End of step       | Postmix                  | No                  |
|                                                                                     |                   | Collect count            | 4                   |
|                                                                                     |                   | Collect time [s]         | 10                  |
|  | Bead Removal      | Washing Plate 3          |                     |
|                                                                                     |                   | Release time, speed      | 00:00:30, Fast      |
|  | Leave             | Tip Plate                |                     |

## Troubleshooting

| Problem                                                                          | Probable cause                                               | Comments and suggestions                                                                                                                                                                                     |
|----------------------------------------------------------------------------------|--------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| low amount of extracted DNA                                                      | insufficient lysis                                           | increase lyses time, but prevent too long lyses time because this also decreases the yield<br>Reduce amount of starting material                                                                             |
|                                                                                  | incomplete elution                                           | increase volume of <b>Elution Buffer</b> / increase time of elution step                                                                                                                                     |
|                                                                                  | low amount of <b>SNAP Solution</b>                           | mix <b>SNAP Solution</b> thoroughly before addition                                                                                                                                                          |
| low concentration of extracted DNA                                               | too much <b>Elution Buffer</b>                               | elute the DNA with a lower volume of <b>Elution Buffer</b> (don't use less than 100 µl)                                                                                                                      |
|                                                                                  | incorrect storage of starting material                       | ensure that storage of starting material is correct<br>avoid repeated freezing and thawing cycles of the material                                                                                            |
| degraded / sheared DNA                                                           | incorrect storage of starting material                       | ensure that the storage of starting material was correct<br>avoid multiple freezing and thawing cycles of the sample material                                                                                |
|                                                                                  | old material                                                 | ensure that the starting material is fresh or stored at appropriate conditions (long-term storage at -20°C)<br>old material often contains degraded DNA                                                      |
| DNA does not perform well in downstream-applications (e.g. real-time PCR or PCR) | ethanol carryover during elution                             | increase drying time for evaporation of ethanol                                                                                                                                                              |
|                                                                                  | salt carryover during elution                                | check the <b>Wash Buffers</b> for salt precipitates. If any precipitates are visible, solve them by carefully warming up to 30°C<br>ensure that the <b>Wash Buffers</b> are equilibrated at room temperature |
| low $A_{260}:A_{280}$ ratio from UV measurement, eluted DNA is brown colored     | small part of the magnetic particles are left in the elution | centrifuge at full speed for 1 min and transfer supernatant to a new tube                                                                                                                                    |

## Appendix

### KingFisher BindIt Software 3.2 or higher versions

BindIt software 3.2 or higher versions were and may be used to create assay files for the KFmL, KF96/KFflex96 or KF-Duo instruments. The provided assay file(s) can either be transferred onto the corresponding workstation(s) or be started directly from within the BindIt software after assay import. Please keep in mind, that assay(s) run from within the BindIt software are not stored in the workstation memory.

**Important:** *Be advised that BindIt SW 3.2 or higher versions use a new unique file extension. Therefore, it is not possible to import assay files created with BindIt 3.2 or higher versions into older BindIt software versions! Please ask your local Thermo Scientific distributor for a software update.*

**Note:** *When creating assay files for usage with KingFisher instruments in combination with Microtiter Deep Well plates (e.g. Thermo Electron), it is essential to use the KingFisher software 3.2 or higher versions for assay development because this software version includes the correct adjustments for the microtiter plate. It is highly recommended to use Thermo Microtiter Deep Well plates with KF96 / KFflex96 / KF-Duo workstations to ensure the best purification result.*

### Minimum system requirements for BindIt Software 3.2 or higher versions

| PC requirements             |                                                                                        |
|-----------------------------|----------------------------------------------------------------------------------------|
| Supported operating systems | MS Windows XP Pro with SP3, Windows Vista SP2, Windows 7                               |
| Disk space                  | 500 MB free disk space                                                                 |
| Processor                   | Intel Pentium $\geq$ 1 GHz                                                             |
| Memory                      | 1 GB RAM                                                                               |
| Serial ports available      | 1 (for KFmL connection)                                                                |
| USB ports available         | 1 (for KF96 / KFflex96 / KFDuo connection)                                             |
| Pointing device             | Mouse or equivalent is required                                                        |
| CD-ROM drive                | 1                                                                                      |
| Monitor / color settings    | XVGA monitor with at least 1024x768 resolution and at least a 16-bit color environment |

If the actual Windows Service Packs are not installed on the corresponding lab computer, they can be downloaded from the Microsoft web pages: <http://www.microsoft.com/>

## **General notes on handling DNA**

### **Nature of DNA**

The length and delicate physical nature of DNA requires careful handling to avoid damage due to shearing and enzymatic degradation. Other conditions that affect the integrity and stability of DNA include acidic and alkaline environments, high temperature, and UV irradiation. Careful isolation and handling of high molecular weight DNA is therefore required to ensure compatibility with various downstream applications. Damaged DNA may perform poorly in applications such as genomic Southern Blotting or long-template PCR.

### **Storage of DNA**

A working stock of DNA can be stored at 2-8°C for several weeks. For long-term storage DNA should be stored at -20°C, but storing at -20°C may cause shearing, particularly if the DNA is exposed to repeated freezing and thawing cycles.

Note that the solution, in which the nucleic acid is eluted in, will affect its stability during storage. Pure water lacks buffering capacity and an acidic pH may lead to acid hydrolysis. Tris or Tris-EDTA buffer contains sufficient buffering capacity to prevent acid hydrolysis.

### **Drying, dissolving and pipetting DNA**

Avoid overdrying of genomic DNA after ethanol precipitation. We highly recommend to air dry than to use a vacuum, although vacuum drying can be used with caution.

Avoid vigorous pipetting. Pipetting of genomic DNA through small tip openings can cause shearing or nicking. One way to decrease shearing of genomic DNA is to use special tips that have wide openings designed for pipetting genomic DNA.

### **DNA yield**

The amount of purified DNA from the plant material depends on sample source, transport conditions, storage and age of the sample.

## Ordering information

|                                               |            |                   |
|-----------------------------------------------|------------|-------------------|
| InviMag <sup>®</sup> Plant DNA Mini Kit /KF96 | 7437300100 | 1 x 96 preps      |
| InviMag <sup>®</sup> Plant DNA Mini Kit /KF96 | 7437300200 | 5 x 96 preps      |
| InviMag <sup>®</sup> Plant DNA Mini Kit/ KFmL | 2437110100 | 15 purifications  |
| InviMag <sup>®</sup> Plant DNA Mini Kit/ KFmL | 2437110200 | 75 purifications  |
| Invisorb <sup>®</sup> DNA Plant HTS 96 Kit/ C | 7037300200 | 2 x 96 preps      |
| Invisorb <sup>®</sup> DNA Plant HTS 96 Kit/ C | 7037300300 | 4 x 96 preps      |
| Invisorb <sup>®</sup> DNA Plant HTS 96 Kit/ C | 7037300400 | 24 x 96 preps     |
| Invisorb <sup>®</sup> Spin Plant Mini Kit     | 1037100200 | 50 purifications  |
| Invisorb <sup>®</sup> Spin Plant Mini Kit     | 1037100300 | 250 purifications |

### Possible suppliers for Isopropanol:

**Carl Roth**  
2-Propanol  
Rotipuran >99.7%, p.a., ACS, ISO  
Order no. 6752

**Applichem**  
2-Propanol für die Molekularbiologie  
Order no. A3928

**Sigma**  
2-Propanol  
Order no. 59304-1L-F

## Ordering information (KingFisher 96 and consumables)

| Cat.no   | Description                                                                                 |
|----------|---------------------------------------------------------------------------------------------|
| 5400500  | KingFisher 96, magnetic particle processor, 100-240V, 50/60Hz (including one magnetic head) |
| 24073430 | KingFisher 96 head for Deep Well Plates                                                     |
| 97002514 | KingFisher 96 tip comb for a PCR magnet head / plates, 8 x 10 pcs / box                     |
| 97002524 | KingFisher 96 tip comb for KF magnets / plates, 10 x 10 pcs / box                           |
| 97002534 | KingFisher 96 tip comb for DWP magnets / DWP 10 x 10 pcs / box                              |
| 97002540 | KingFisher 96 KF plate (200 µl) 48 plates / box                                             |
| 95040450 | Microtiter deep well 96 plate (2 ml), 50 plates / box                                       |



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