Instruction for InviMag® Plant DNA Mini Kit/ KF96 / KFflex96

The **InviMag® Plant DNA Mini Kit/ KF96/ KFflex96** is the ideal tool using the **InviMag®** technology for isolation and purification of DNA from max. 100 mg plant material and food of plant origin.

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

Content

Kit contents of InviMag® Plant DNA Mini Kit/ KF96/ KFflex96

Store the **SNAP Solution** at 4 ℃
Store lyophilized **Proteinase K** at 2 – 8 ℃
Store diluted **Proteinase K** at –20 ℃
Store all other kit components at room temperature!

	96 extractions	5 x 96 extractions
Catalogue Number	7437300100	7437300200
Lysis Buffer P	50 ml	210 ml
Proteinase K working solution	2 x 1.5 ml	10.5 ml
Binding Buffer P	30 ml	110 ml
SNAP Solution	2 x 1.1 ml	10.5 ml
Wash Buffer I	80 ml final volume 160 ml	3 x 80 ml final volume 3 x 160 ml
Wash Buffer II	60 ml final volume 200 ml	5 x 60 ml final volume 5 x 200 ml
Elution Buffer D	15 ml	60 ml
2.0 ml Deep Well Plate	4	20
KF 96 Tip Comb for DW magnets	1	5
200 µl Elution Plate*	2	10
Sealing Foils	2	10
Manual	1	1
Initial steps	dilute **Proteinase K** by addition of 1.5 ml of ddH_2O, mix thoroughly and store like described below! add 80 ml of 96 - 100 % ethanol to the bottle **Wash Buffer I** add 140 ml of 96 - 100 % ethanol to the bottle **Wash Buffer II** mix **Wash Buffer** thoroughly and always keep the bottles firmly closed	dilute **Proteinase K** by addition of 10.5 ml of ddH_2O, mix thoroughly and store like described below! add 80 ml of 96 - 100 % ethanol to the bottle **Wash Buffer I** add 140 ml of 96 - 100 % ethanol to the bottle **Wash Buffer II** mix **Wash Buffer** thoroughly and always keep the bottles firmly closed

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

Symbols

 manufacturer

 lot number

 catalogue number

 date of manufacture

 expiry date

 consult operating instructions

 temperature limitation

 do not reuse

Storage

All buffers and kit contents of the **InviMag® Plant DNA Mini Kit/ KF96/ KFflex96**, except Proteinase K and SNAP Solution should be stored at room temperature and are stable for at least 12 months under these conditions.
The lyophilized Proteinase K can be stored at 2 – 8°C.

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

Proteinase K: Dissolved Proteinase K must be stored at –20 °C. Dividing the Proteinase K into aliquots and storage at –20 °C is recommended **.**

SNAP Solution should be stored at 2 – 8°C **.**.

Wash Buffer I and II
Wash Buffer charged with ethanol should be stored at room temperature and should be appropriate sealed. If there are any precipitates within the provided solutions solve these precipitates by careful warming up to 30 °C.

Quality control

Invitek guarantees the correct function of the **InviMag® Plant DNA Mini Kit/ KF96/ KFflex96** for applications as described in the manual. In accordance with Invitek's certified QM-System each component of the **InviMag® Plant DNA Mini Kit/ KF96/ KFflex96** was tested against predetermined specifications to ensure consistent product quality.

If you have any questions or problems regarding any aspects of **InviMag® Plant DNA Mini Kit/ KF96/ KFflex96** or other Invitek products, please do not hesitate to contact us.

For technical support or further information please contact: Tel.: +49 (0) 30 9489 2894, 2910 in Germany and from foreign countries Tel.: +49 (0) 30 9489 2907 or your local distributor (see page 20)

Intended use

The **InviMag® Plant DNA Mini Kit/ KF96/ KFflex96** has been designed for a semi automated preparation of genomic DNA for up to 100 mg plant or food (plant origin) material using magnetic beads and the KingFisher 96/ KingFisher Flex.

The whole process is based on the patented **InviMag® technology**, for isolation of genomic DNA by binding the nucleic acid onto magnetic particles without chaotropic buffer components.

For reproducible and high yields an appropriate sample storage is essential.

The product is intended for use by professional users 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 modification of DNA followed by signal detection or amplification. Any results generated using the sample preparation procedure in conjunction with any downstream assay should be interpreted with regard to other laboratory findings.

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

Product use limitation

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

The included chemicals and magnetic beads are once only useable.

When differing the starting material or the flow trace, no guarantee in operability is issued.

The user is responsible to validate the performance of the Invitek kits for any particular use, since the performance characteristics of our kits have not been validated for any specific application. Invitek kits may be used in clinical diagnostic laboratory systems after the laboratory has validated the complete diagnostic system as required by CLIA' 88 regulations in the U.S. or equivalents in other countries.

All products sold by Invitek are subjected to extensive quality control procedures (according to EN ISO 9001-2000 and EN ISO 13485-2003) and are warranted to perform as described when used correctly. Any problems should be reported immediately.

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

The kit contents are unfit for consumption.

Safety information

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. Heed the legal requirements for working with biological material!

For more information, please consult the appropriate material safety data sheets (MSDS). They are available online in convenient and compact PDF format at **www.invitek.de** under each Invitek kit and whose kit component.

If the buffer bottles are damaged or leaking, wear gloves and protective goggles when discarding the bottles in order to avoid any injuries.

Invitek has not tested the liquid waste generated by the **InviMag® Plant DNA Mini Kit/ KF96/ KFflex96** procedures for residual infectious materials. Contamination of the liquid waste with residual infectious materials is highly unlikely, but cannot be exclude completely. Therefore, liquid waste have be considered infectious and be handled and discarded according to local safety regulation.

Below European Community risk and safety phrases for the components of the **InviMag® Plant DNA Mini Kit/ KF96/ KFflex96** to which they apply, are listed.

Lysis Buffer P

 **Xi**

irritant

R36 S24

Proteinase K

 **Xn**

harmful

R36/37/38-42 S2-22-24-26-36/37

Binding Buffer P

 **Xi;**  **F** contains 2-propanol

irritant; highly flammable

R11-36-67 S7-16-24/25-26

Wash Buffer I

 **Xn**

harmful

R20/21/22-32-52/53 S2-13-61

R11:	highly flammable
R20/21/22:	harmful by inhalation, in contact with skin and if swallowed
R32:	contact with acids liberates very toxic gas
R36:	irritating to eyes
R36/37/38:	irritating to eyes, respiratory system and skin
R42:	may cause sensitization by inhalation.
R52/53:	harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment
R67:	vapors may cause drowsiness and dizziness

S2:	keep out of the reach of children
S7:	keep container tightly closed
S13:	keep away from food, drink and animal feeding stuffs
S16:	keep away from sources of ignition - No smoking
S22:	do not breath dust
S24/25:	avoid contact with skin and eyes
S24:	avoid contact with skin
S26:	in case of contact with eyes, rinse immediately with plenty of water seek medical advice
S36/37:	wear suitable protective clothing and gloves
S61:	avoid release to the environment. Refer to special instructions/safety data sheet

Emergency medical information in English and German can be obtained 24 hours a day from:

Poison Information Center Freiburg, Germany: Tel.: +49 761 19240

Product characteristic of the InviMag® Plant DNA Mini Kit/ KF96/ KFflex96

Starting material	Yield	Time	Ratio
up to 100 mg plant material	5 - 25 µg; depends on the kind of starting material	about 20 - 25 min after lysis	A_{260}:A_{280} 1.8-2.0

The **InviMag® Plant DNA Mini Kit/ KF96/ KFflex96** has been designed for a semi automated preparation of genomic DNA for up to 100 mg of plant sample using magnetic beads and the KingFisher ml. The isolation process is based on a patented technology, the **InviMag®** technology.

The DNA isolation process is based on the interaction of nucleic acids with coated magnetic particles under adapted buffer conditions. The KingFisher 96 performs all steps of the DNA purification procedure automatically without any user intervention. The procedure requires minimal interaction by the user. Sample cross contamination and reagent cross-over is effectively eliminated by this automated purification process.

The KingFisher® instruments use magnetic rods to move the DNA-binding magnetic particles through the various purification phases: binding-washing-elution. The volume of buffers and other liquids necessary for DNA isolation is reduced to a minimum. Eliminating the direct liquid handling and increasing the automation level result in a fast, reliable and robust technique. The overall efficiency speed up the procedure.

To realize an efficient lysis and highest yields, the plant samples are first mechanically disrupted, followed by lysis in an optimized buffer containing **Proteinase K**. Cell debris is removed by centrifugation step. The cleared lysates are transferred to the subsequent automatic purification procedure based on magnetic beads. The DNA binds to magnetic particles, followed by washing steps and the final elution. The procedure requires minimal interaction by the user, allowing safe handling of samples. The procedures are designed to avoid sample-to-sample cross contamination.

The purified high quality DNA is ready to use for subsequent downstream applications (see below) or can be stored at –20 ℃ for subsequent us e.

- PCR*
- Genotyping
- Restriction Digestion

No toxic or hazardous chemicals like chaotropic components are used.

For the isolation of genomic DNA in a large scale using magnetic particle from DNA binding in high-through- put format, Invitek offers the **InviMag® Plant DNA Midi Kit/ KFflex 24** for use on the KingFisher Flex 24 (see ordering information)

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

For further information please contact: Tel.: +49 (0) 30 9489 2894, 2910 in Germany and from foreign countries Tel.: +49 (0) 30 9489 2907 or your local distributor (see page 20).

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

Principle and procedure

The **InviMag® Plant DNA Mini Kit/ KF96/ KFflex96** procedure comprises following steps:

- lysis of sample material
- binding the genomic DNA to the magnetic beads
- washing and elimination of ethanol
- elution of genomic DNA

After lysis and clearing the DNA binds to the magnetic beads, after that contaminations, metabolites and enzyme inhibitors are efficiently removed during the following three wash steps and highly purified DNA is eluted in Elution Buffer D or water.

Sampling and storage of starting material

Harvested plant samples can be stored at room temperature for 2 – 3 hours, for short time storage (up to one week) samples may be stored at 4°C. For long term storage, we recommend freezing samples at –20°C or –80°C. Multi ple thawing and freezing before isolating the DNA should be avoided.

Procedure

Lysis
Samples are disrupted by using a mixer mill, or beads or by mortar and pistill. The disrupted lysate is lysed under denaturing non-chaotropic conditions at elevated temperatures in the presence of **Lysis Buffer P** and **Proteinase K** on the platform of the KingFisher 96 under permanent mixing. In case of a large number of samples the preparation of a master mixture with a volume 5% greater than that required for the processing of all samples is recommended. Mix the master mix carefully prior to use!

Binding of the DNA
After addition of **Binding Buffer P** and **SNAP Solution** to the lysate, optimal binding conditions will be adjusted and the genomic DNA is adsorbed to the beads.

Removing residual contaminants
Contaminants are efficiently washed away using **Wash Buffer**, while the DNA remains bound to the magnetic beads

Elution
The DNA is eluted from the beads using 200 µl **Elution Buffer D**. The eluted DNA is ready for use in different subsequent downstream applications e.g.

- Southern blotting
- PCR, RAPD, AFLP analysis
- microsatellite analysis
- genotyping
- enzymatic restriction digestion

Yield and quality of genomic DNA

The amount of purified DNA in the **InviMag® Plant DNA Mini Kit/ KF96/ KFflex96** procedure from plant material depends on sample source, transport conditions, storage and age of the sample.
Yield and quality of isolated genomic DNA is suitable for any detection system.

Important points before starting a protocol

After receiving the kit, check the kit components for damage. If buffer bottles are damaged, contact the Invitek Technical Services or your local distributor. In case of liquid spillage, refer to “Safety information” (page 6). Do not use damaged kit components, since their use may lead to poor kit performance.

- always change pipet tips between liquid transfer. To avoid cross-contamination, we recommend the use of aerosol-barrier pipet tips
- all centrifugation steps are carried out at room temperature
- when working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles
- discard gloves if they become contaminated
- 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. Where necessary, gently mix and re-dissolve any precipitates by incubation at 30°C. Swirl gently to avoid foaming.
Lysis Buffer P, Binding Buffer P and **Elution Buffer D** are ready to use.
Add the needed µl ddH_2O to reaction tube with **Proteinase K**. Vortex for 5 s and store diluted **Proteinase K** at –20°C.

1 x 96 DNA-extractions:
dilute **Proteinase K** by addition of 1, 5 ml of ddH_2O, mix thoroughly and store like described on page 2 add 80 ml of 96 - 100 % ethanol to the bottle **Wash Buffer I** add 140 ml of 96 - 100 % ethanol to the bottle **Wash BufferII**. mix thoroughly and keep the bottle always firmly closed
5 x 96 DNA-extractions:
dilute **Proteinase K** by addition of 10,5 ml of ddH_2O, mix thoroughly and store like described on page 2 add 80 ml of 96 - 100 % ethanol to the bottle **Wash Buffer I** add 140 ml of 96 - 100 % ethanol to the bottle **Wash Buffer II** mix thoroughly and keep the bottle always firmly closed divide the SNAP Solution according your need

Reagents and equipment to be supplied by user

- measuring cylinder (250 ml)
- pipette and pipette tips
- disposable gloves
- reaction tubes (1.5 ml or 2.0 ml)
- ddH_2O
- vortexer
- 96-100% Ethanol

Important indications for the InviMag® Plant DNA Mini Kit/ KF96/ KFflex96

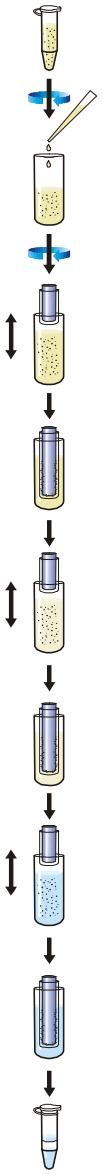
The kit can also purify RNA besides DNA. For the elimination of RNA (if necessary) add 20 µl RNase A (10 mg/ ml) to **Binding Buffer P** in Binding Plate.

Scheme of the InviMag® Plant DNA Mini Kit/ KF96/ KFflex96

Please read protocols prior the start of the preparation carefully

Add needed amount of ddH_2O or PBS to adjust the sample volume to 200 µl.
Transfer each sample in a well of the 2.0 ml Deep Well Plate.
Prefill all plates of the KingFisher 96 with needed buffers and the appropriate buffer volumes.

Tip Plate:	Place in the KF 96 Tip Comb for DW magnets on a Tip Plate*
Binding Plate:	Add **200 µl Binding Buffer P** & **20 µl SNAP Solution** to the lysate
Washing Plate_1:	Add **800 µl Wash Buffer I** to a 2.0 ml Deep Well Plate
Washing Plate_2:	Add **800 µl Wash Buffer II** to a 2.0 ml Deep Well Plate
Washing Plate_3:	Add **800 µl Wash Buffer II** to a 2.0 ml Deep Well Plate
Elution Plate:	Pipet **100 µl Elution Buffer D** to a 200 µl Elution Plate



Mix sample with 400 µl **Lysis Buffer P** and 20 µl **Proteinase K**. Mix by vortexing for 5 s and incubate for 30 min at 65 ℃ under continuously mixing or shaking

During lysis time prefill all plates strips with needed buffer and the appropriate buffer volume

Addition of 200 µl **Binding Buffer P** and 20 µl **SNAP Solution** to the lysate

DNA binds to magnetic particles

Magnetic separation

Washing of the particle fixed genomic DNA

Magnetic separation

Elution of genomic DNA

Magnetic Separation

Pure DNA

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

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

Please read the instructions carefully and conduct the prepared procedure.

1. Homogenization of the starting material

Homogenize up to 100 mg of starting material e.g. by a pestle under liquid nitrogen. Commercially available equipment for homogenization also can be used.

2. Sample Lysis

Transfer homogenized starting material into a 1.5 ml Reaction Tube (not provided). Add 400 µl of Lysis Buffer P and 20 µl of Proteinase K into the 1.5 ml Reaction Tube.

Vortex the tube for 5 s and incubate the sample at 65°C for app. 30 min under continuously shaking (e.g. by using a thermo mixer).

After lysis time centrifuge the 1.5 ml Reaction tube at max. speed for 1 minute to pellet down the unlysed material. Transfer the supernatant from the lysed sample carefully to the Lysis Plate:.

Note: *The kit can also purify RNA besides DNA. For the elimination of RNA (if necessary) add 20 µl RNase A (10 mg/ml) to Binding Buffer P in the Lysis Plate.*

3. Preliminary Steps to process the samples onto the KingFisher System

Note: *Before starting the purification process with the KingFisher instrument please carefully read the user manual!*

During the sample lysis prefill the plates with the following Buffers respectively. Please avoid evaporation of the prefilled buffer components. In case of long term use, protect the plate from drying-out by use of sealing foil or parafilm!

Note: *Resuspend the magnetic particles (SNAP Solution A) thoroughly before use!*

Lysis Plate:	Add 400 µl lysed sample, 200 µl Binding Buffer P and 20 µl SNAP Solution
Washing_Plate_1:	Add 800 µl Wash Buffer I
Washing_Plate_2:	Add 800 µl Wash Buffer II
Washing_Plate_3:	Add 800 µl Wash Buffer II
Elution Plate:	Add 100 µl Elution Buffer D

Insert the tube strip tray to the instrument and insert the tip combs into the slots.

Close the front lid and start the process by selecting protocol **"InviMag Plant DNA Mini Kit KF96" (KF96)** or **"InviMag Plant DNA Mini Kit KFflex 96" (KFflex 96)** using arrow keys and press START.

The following extraction steps running automatically on the KingFisher System!

1. Binding of the DNA

Automatically sample mixing for 4 min. SNAP separation. Transfer of the SNAPs to the Washing Plate 1.

2. First Washing

Automatically sample mixing for 150 s. SNAP separation. Transfer of the SNAPs to the Washing Plate 2.

3. Second Washing

Automatically sample mixing for 90 s. SNAP separation. Transfer of the SNAPs to the Washing Plate 3.

4. Third Washing

Automatically sample mixing for 90 s. SNAP separation. Transfer of the SNAPs outside Washing Plate 3.

5. Drying

Drying of the SNAP outside Washing Plate 3 for 3 minutes. Transfer of the Beads to the Elution Plate.

6. Elution of the DNA

Incubation of SNAPs in the elution buffer for 10 minutes by continuous mixing. SNAP separation. The SNAP will automatically be removed in Washing Plate 3 (disposal).

Important Notes:

1. *After finishing the extraction protocol, the Eluion Plate contains the extracted DNA. Store the DNA under adequate conditions. We recommend to transfer the extracted DNA into the 1.5 ml Receiver Tubes for further storage and freeze the DNA at –20 °C.*
2. *If the extracted DNA contains carryover of magnetic particle, transfer the DNA into a 1.5 ml reaction tube, centrifuge at maximum speed for 1 minute and pipet the DNA into a new tube.*

The eluted DNA is ready for use in different downstream applications.

Eluted DNA stored at 4–8 °C is stable for several weeks or stored at –20 °C for more than 5 years.

For self programming of the KingFisher 96 System (program "InviMag Plant DNA Mini Kit KF96")

[PROTOCOL PROPERTIES]

Name = InviMAG_Plant_DNA-Mini-Kit_KF96
Protocol template version = 2.6.0
Instrument type = KingFisher 96
Creator = Invitek GmbH
Description = KF96 protocol (Thermo Electron) for isolation of genomic DNA from Plants
Plate layouts = Tip Plate, Elution Plate, Binding Plate, Washing Plate_1, Washing Plate_2, Washing Plate_3

[PLATE LAYOUTS]

TIP PLATE

Plate type = KingFisher 96 plate
Plate change message = Insert Tip Plate
A:
- EMPTY

ELUTION PLATE

Plate type = KingFisher 96 plate
Plate change message = Insert Elution
A:
- volume = 100, name = Elution Buffer P

BINDING PLATE

Plate type = KingFisher 96 DW plate
Plate change message = Insert Bind plate
A:
- volume = 420, name = Sample
- volume = 200, name = Binding Buffer P
- volume = 20, name = SNAP Solution

WASHING PLATE 1

Plate type = KingFisher 96 DW plate
Plate change message = Insert Wash_1
A:
volume = 600, name = Wash Buffer I

WASHING PLATE 2

Plate type = KingFisher 96 DW plate
Plate change message = Insert Wash_2
A:
- volume = 800, name = Wash Buffer II

WASHING PLATE 3

Plate type = KingFisher 96 DW plate
Plate change message = Insert Wash_3
A:
- volume = 800, name = Wash Buffer II

[STEPS]

HEATING

Step parameters
Name = Heating
Plate = Binding Plate

Beginning of step:
Release = Yes, time = 0s, speed = Medium

Heating parameters:
Heating time = 30min 0s
Preheat = Yes
Temperature = 65
During heating = Mix,
Postmix time = 0s, speed = Slow

End of step:
Collect beads = Yes, count = 3

PAUSE

Step parameters
Name = Reagent Distribution
Plate = Binding Plate
Message = Add Beads and Buffer
Dispense:Buffer and Beads, volume=220ul

BINDING

Step parameters
Name = Binding
Plate = Binding Plate

Beginning of step:
Release = Yes, time = 10s, speed = Medium

Bind parameters:
Bind time = 5min 0s, speed = Slow

End of step:
Collect beads = Yes, count = 3

WASHING STEP 1

Step parameters
Name = Washing Step 1
Plate = Washing Plate_1

Beginning of step:
Release = Yes, time = 30s, speed = Medium

Wash parameters:
Wash time = 2min 0s, speed = Medium

End of step:
- Collect beads = Yes, count = 3

WASHING STEP 2

Step parameters
Name = Washing Step 2
Plate = Washing Plate_2

Beginning of step:
Release = Yes, time = 30s, speed = Medium

Wash parameters:
Wash time = 1min 0s, speed = Medium

End of step:
Collect beads = Yes, count = 3

WASHING STEP 3

Step parameters
Name = Washing Step 3
Plate = Washing Plate_3

Beginning of step:
Release = Yes, time = 30s, speed = Medium

Wash parameters:
Wash time = 1min 0s, speed = Medium

End of step:
Collect beads = Yes, count = 3

DRYING

Step parameters
Name = Drying
Plate = Washing Plate_3
Dry time = 3min 0s
Tip position = Outside well

ELUTION

Step parameters
Name = Elution
Plate = Elution Plate

Beginning of step:
Release = Yes, time = 10s, speed = Medium

Elution parameters:
Elution time = 10min 0s, speed = Slow

Heating parameters:
Heating time = 10min 0s
Preheat = No
Temperature = 70
During heating = Mix
Postmix time = 0s, speed = Medium

REMOVE BEADS

Remove beads = Yes, collect count = 3,
disposal plate = Washing Plate_3

For self programming of the KingFisher Flex 96 System (program “InviMag Plant DNA Mini Kit KFflex 96”)

[PROTOCOL PROPERTIES]

Name = InviMAG Plant DNA Mini-Kit KFflex 96
Protocol template version = 3.1
Instrument type = KingFisher Flex 96
Creator = Invitek GmbH
Description = KFflex 96 protocol (Thermo Electron) for isolation of genomic DNA from Plants
Plate layouts = Tip Plate, Elution Plate, Binding Plate, Washing Plate_1, Washing Plate_2, Washing Plate_3

[PLATE LAYOUTS]

TIP PLATE
Plate type = KingFisher 96 plate
Reagents: <empty>

LYSIS PLATE
Plate type: KingFisher 96 DW plate

Reagents:
Name: Binding Buffer P
Volume [µl]: 200
Type: Reagent

Name: SNAP Solution
Volume [µl]: 20
Type: Reagent

Name: Sample mixture
Volume [µl]: 420
Type: Sample

WASHING PLATE 1
Plate type: KingFisher 96 DW plate

Reagents:
Name: Wash Buffer I
Volume [µl]:600
Type: Reagent

WASHING PLATE 2
Plate type: KingFisher 96 DW plate

Reagents:
Name: Wash Buffer II
Volume [µl]: 800
Type: Reagent

WASHING PLATE 3
Plate type: KingFisher 96 DW plate
Reagents:
Name:Wash Buffer II
Volume [µl]: 800
Type: Reagent

ELUTION PLATE
Plate type: KingFisher 96 plate
Reagents:
Name: Elution Buffer P
Volume [µl]: 100
Type: Reagent

[STEPS]

TIP PICKUP
Pick-Up plate: 96 DW tip comb (Tip Plate)
Leave plate: 96 DW tip comb (Tip Plate)

LYSIS
Plate: Lysis Plate
Beginning of step:
Precollect: No
Release beads: Yes

Mixing/heating parameters:
Heating temperature [°C]: 65
Preheat: Yes
Mixing time [hh:mm:ss]: 00:30:00
Mixing speed: Medium

End of step:
Postmix: No
Collect beads: No

PAUSE STEP
Plate: Lysis Plate

Message: Add Binding + Beads
Dispensing volume [µl]: 220
Reagent name: Beads + Binding Buffer

BINDING
Plate: Lysis Plate

Beginning of step:
Precollect: No
Release time [hh:mm:ss]: 00:00:10
Release speed: Medium

Mixing/heating parameters:
Heating during mixing: No
Mixing time [hh:mm:ss]: 00:05:00
Mixing speed: Slow

End of step:
Postmix: No
Collect count: 3

Collect time [s]: 2

WASHING STEP 1
Plate: Washing Plate 1

Beginning of step:
Precollect: No
Release time [hh:mm:ss]: 00:00:30
Release speed: Medium

Mixing/heating parameters:
Heating during mixing: No
Mixing time [hh:mm:ss]: 00:02:00
Mixing speed: Medium

End of step:
Postmix: No
Collect count: 2
Collect time [s]: 2

WASHING STEP 2
Plate: Washing Plate 2

Beginning of step:
Precollect: No
Release time [hh:mm:ss]: 00:00:30
Release speed: Medium

Mixing/heating parameters:
Heating during mixing: No
Mixing time [hh:mm:ss]: 00:01:00
Mixing speed: Medium

End of step:
Postmix: No
Collect count: 2
Collect time [s]: 2

WASHING STEP 3
Plate: Washing Plate 3

Beginning of step:
Precollect: No
Release time [hh:mm:ss]: 00:00:30
Release speed: Medium

Mixing/heating parameters:
Heating during mixing: No
Mixing time [hh:mm:ss]: 00:01:00
Mixing speed: Medium

End of step:
Postmix: No
Collect count: 2
Collect time [s]: 2

DRYING
Plate: Washing Plate 3

Dry time [hh:mm:ss]: 00:03:00
Tip position: Outside well/tube

ELUTION
Plate: Elution Plate

Beginning of step:
Precollect: No
Release time [hh:mm:ss]: 00:00:30
Release speed: Slow

Mixing/heating parameters:
Heating temperature [°C]: 70
Preheat: Yes
Mixing time [hh:mm:ss]: 00:10:00
Mixing speed: Slow

End of step:
Postmix: No
Collect count: 3
Collect time [s]: 2

REMOVE BEADS
Plate: Washing Plate 3

Release time [hh:mm:ss]: 00:00:30
Release speed: Fast

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 decrease yield Reduce amount of starting material
	incomplete elution	take higher volume of **Elution Buffer D**, be sure you pipet the **Elution Buffer D** with the right amount to the right position
	low amount of **SNAP Solution**	mix **SNAP Solution** thoroughly before pipeting to the KingFisher tube
low concentration of extracted DNA	too much **Elution Buffer D**	elute the DNA with lower volume of **Elution Buffer D**
	incorrect storage of starting material	ensure that the storage of starting material was correctly Avoid repeated thawing of the material
degraded or sheared DNA	incorrect storage of starting material	ensure that the storage of starting material was correctly Avoid thawing of the material
	old material	ensure that the starting material is fresh or stored under appropriate condition (for long time storage at –20 ℃) avoid thawing and freezing of the material 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 removing of ethanol
	salt carryover during elution	check up the **Wash Buffers** for salt precipitates. If there are any precipitates, solve these precipitates by careful warming ensure that the **Wash Buffers** are 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 down at full speed for 1 min and transfer supernatant to a new tube

Appendix

KingFisher Software Version 2.6 and 3.1

The KingFisher Software 2.6 was used for the creation of the KingFisher 96 protocols whereas the Software 3.1 was used for creation of KingFisher Flex 96 run files. The user can either transfer the protocol onto the workstation or run the the protocol directly from the software. Be aware that directly run protocols are not stored in the workstation memory.
If you don´t have the correct KingFisher software installed on your computer, please call your local ThermoFisher distributor for an update.

Note: ***Please keep in mind that software version 2.6 and 3.1 are not compatible! It is not possible to run a procotol created in version 2.6 under version 3.1 and vice versa!***

Minimal PC Requirements for KingFisher Software 2.6 and 3.1

PC requirements	
Interface	Serial communication port via a RS-232 full duplex interface
Supported operating systems	Microsoft Windows 2000
Disk space	500 MB free disk space
Processor	Intel Pentium ≥ 700 Mhz recommended
Memory	220 MB RAM recommended
Serial ports available	1
Pointing device	Mouse or equivalent is necessary
CD-ROM drive	1
Monitor / color settings	SVGA monitor with at least 1024 x 768 resolution and at least a 16-bit color environment
Service packs installed	Microsoft Windows 2000: Service Pack 4 (or greater) Microsoft Windows XP Professional: Service Pack 2 (or greater)
Browser	Microsoft Internet Explorer 6.0 (or greater) installed

If you do not have the correct Service Packs installed, you can download them 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 necessary to ensure compatibility with various downstream applications. Damaged DNA could perform poorly in applications such as genomic Southern blotting, long-template PCR.

Storage of DNA

A working stock of DNA can be stored at 2–4 ℃ for several weeks. For long term storage DNA should be stored at –20 ℃, but storing at –20 ℃ can cause shearing, particularly if the DNA is exposed to repeated freeze-thaw cycles.

Note that the solution in which the nucleic acid is eluted in will affect it's 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 genomic DNA after ethanol precipitation. It is better to let it air dry than to use a vacuum, although vacuum drying can be used with caution.
Avoid vigorous pipeting. Pipeting genomic DNA through small tip openings causes shearing or nicking. One way to decrease shearing of genomic DNA is to use special tips that have wide openings designed for pipeting 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.

Order information

InviMag® Plant DNA Mini Kit/ KF96/ KFflex96	7437300100	1 x 96 preps
InviMag® Plant DNA Mini Kit/ KF96/ KFflex96	7437300200	5 x 96 preps
InviMag® Plant DNA Mini Kit/ KFmL	2437110100	15 purifications
InviMag® Plant DNA Mini Kit/ KFmL	2437110200	75 purifications
Invisorb® DNA Plant HTS 96 Kit/ C	7037300200	2 x 96 preps
Invisorb® DNA Plant HTS 96 Kit/ C	7037300300	4 x 96 preps
Invisorb® DNA Plant HTS 96 Kit/ C	7037300400	24 x 96 preps
Invisorb® DNA Plant HTS 96 Kit/ V	7037310100	1 x 96 preps
Invisorb® DNA Plant HTS 96 Kit/ V	7037310200	2 x 96 preps
Invisorb® DNA Plant HTS 96 Kit/ V	7037310300	4 x 96 preps
Invisorb® DNA Plant HTS 96 Kit/ V	7037310400	24 x 96 preps
Invisorb® Spin Plant Mini Kit	1037100200	50 purifications
Invisorb® Spin Plant Mini Kit	1037100300	250 purifications
Invisorb® Spin Plant Midi Kit	1037110200	25 purifications
Invisorb® Spin Plant Midi Kit	1037110300	50 purifications

Order 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 plate
97002514	KingFisher 96 tip comb for PCR magnets, 8 x 10 pcs / box
97002524	KingFisher 96 tip comb for KF magnets, 10 x 10 pcs / box
97002534	KingFisher 96 tip comb for DW magnets 10 x 10 pcs / box
97002540	KingFisher 96 KF plate (200ul) 48 plates / box
95040450	Microtiter deep well 96 plate, 50 plates/box

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