stratec molecular

User manual
RTP® Mycobacteria Kit

for purification of total DNA from mycobacteria from sputum, bronchial lavage and tissue biopsies

REF 101033220x

STRATEC Molecular GmbH, D-13125 Berlin

Instruction for the RTP® Mycobacteria Kit

The **RTP® Mycobacteria Kit** allows rapid and efficient isolation of high quality DNA from Mycobacteria species from sputum or bronchalveolar sample or tissue biopsies. The **RTP® Mycobacteria Kit** combines an efficient pre-analytical sample processing with the efficient isolation of bacterial DNA by binding of bacterial DNA onto a Spin Filter surface by a patented technology without chaotropic components.

The kit is neither validated for the isolation of genomic DNA from cell free body fluids, like cerebrospinal fluid, synovial fluid or for DNA from stool sample, parasites or the purification of total RNA.

The application of the kit for isolation and purification of viral DNA has not been evaluated.

Compliance with EU Directive 98/79/EC on *in vitro* medical devices.

Not for in-vitro diagnostic use in countries where the EU Directive 98/79/EC on in vitro medical devices is not recognized.

Trademarks: Invisorb®, RTP®, Eppendorf®. 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® technology is covered by patents and patent applications: US 6,110363, US 6,043,354, US 6,037,465, EP 0880535, WO 9728171, WO 9534569, EP 0765335, DE 19506887, DE 10041825.2, WO 0034463.

Invisorb® and RTP® 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 RTP® Mycobacteria Kit

Store all kit components at room temperature (RT)!
Store dissolved Proteinase K at –20°C!

	5 extractions	50 extractions	250 extractions
Catalog No	1033220100	1033220200	1033220300
Extraction Tubes L	5	50	5 x 50
NAC Buffer	2 ml	15 ml	60 ml
Resuspension Buffer R	2 x 2 ml	30 ml	120 ml
Binding Solution (fill with 99.7% Isopropanol)	3 x 1 ml (ready to use)	empty bottle (final volume 30 ml)	empty bottle (final volume 120 ml)
Proteinase K	for 250 µl working solution	for 1 ml working solution	for 5 x 1 ml working solution
Wash Buffer I	15 ml (ready to use)	30 ml (final volume 60 ml)	80 ml (final volume 160 ml)
Wash Buffer II	15 ml (ready to use)	18 ml (final volume 60 ml)	2 x 45 ml (final volume 2 x 150 ml)
Elution Buffer	2 ml	3 x 2 ml	30 ml
RTA Spin Filter Set	5	50	5 x 50
1.5 ml Receiver Tubes	5	50	5 x 50
Manual	1	1	1
Initial steps	Dilute the **Proteinase K** by addition of 250 µl ddH_20, mix thoroughly and store like described below!	Dilute the **Proteinase K** by addition of 1 ml ddH_20, mix thoroughly and store like described below! Fill 30 ml 99.7% **Isopropanol** (molecular biologic grade) into the empty bottle Add 30 ml of 96-100% ethanol to the bottle **Wash Buffer I**, mix thoroughly and keep the bottle always firmly closed! Add 42 ml of 96-100% ethanol to the bottle **Wash Buffer II**, mix thoroughly and keep the bottle always firmly closed!	Dilute the Proteinase K by addition of 1 ml ddH_20, mix thoroughly and store like described below! Fill 120 ml 99.7% **Isopropanol** (molecular biologic grade) into the empty bottle Add 80 ml of 96-100% ethanol to the bottle **Wash Buffer I**, mix thoroughly and keep the bottle always firmly closed! Add 105 ml of 96-100% ethanol to the bottle **Wash Buffer II,** mix thoroughly and keep the bottle always firmly closed!

Symbols

lot number

catalog number

date of manufacture

expiry date

consult operating instructions

temperature limitation

do not reuse

Storage

The **RTP® Mycobacteria Kit** including the **Extraction Tubes L** (incl. **Lysis Buffer**, **Proteinase K**, **Carrier RNA, Lysozyme** and **Internal Control DNA**) should be stored dry, at room temperature (RT) and is stable for at least 12 months under these conditions.

Wash Buffer I and **Wash Buffer II** charged with ethanol should be appropriately sealed and stored at room temperature.

Dividing the **Proteinase K** into aliquots and storage at – 20°C is recommended. Storage at –20°C will prolong the activity, but repeating freezing and thawing will reduce the activity dramatically.

Before every use make sure that all components have room temperature. If there are any precipitates within the provided solutions dissolve these precipitates by carefully warming up to room temperature.

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

Quality control and product warranty

STRATEC Molecular warrants the correct function of the **RTP® Mycobacteria Kit** 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 **RTP® Mycobacteria Kit** 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 **RTP® Mycobacteria Kit** 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 **RTP® Mycobacteria Kit** allows rapid and efficient isolation of high quality DNA from Mycobacteria species from sputum or bronchalveolar sample or tissue biopsies using the **RTP® technology**.

This kit technology yields mycobacterial DNA from different human samples that is free of proteins, nucleases and other impurities and is ready to use for different downstream applications, such as PCR, quantitative PCR and others.

The purified DNA can be used for in-vitro diagnostic analysis only.

THE PRODUCT IS INDENTED FOR USE BY PROFESSIONAL USERS 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 DNA 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 diagnostic results, adequate controls for downstream applications should be used.

The kit is in compliance with EU Directive 98/79/EC on in vitro medical devices. But it is not for in-vitro diagnostic use in countries where the EU Directive 98/79/EC on in vitro medical devices is not recognized.

Product use limitation

The kit is neither validated for the isolation of genomic DNA from cell free body fluids, like cerebrospinal fluid, synovial fluid or for DNA from stool sample, parasites or the purification of total RNA.

The application of the kit for isolation and purification of viral DNA has not been evaluated.

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 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 **RTP® Mycobacteria Kit** procedures for residual infectious materials. Contamination of the liquid waste with residual infectious materials is highly unlikely, but cannot be excluded completely. Therefore, liquid waste must be considered infectious and be handled and discarded according to local safety regulations.

European Community risk and safety phrases for the components of the **RTP® Mycobacteria Kit** to which they apply are listed below as follows:

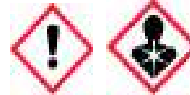
Wash Buffer I

warning
H302-312-332-412 EUH032 P273

Extraction Tube L

danger
H302-314 EUH208 P260-280-305-351-338-310

Proteinase K



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

H319:	Causes serious eye irritation.
H302:	Harmful if swallowed.
H312:	Harmful in contact with skin.
H332:	Harmful if inhaled.
H412:	Harmful to aquatic life with long lasting effects.
EUH032:	Contact with acids liberates very toxic gas.
H314:	Causes severe skin burns and eye damage.
EUH208:	Contains Proteinase K. May produce an allergic reaction.
H315:	Causes skin irritation.
H334:	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335:	May cause respiratory irritation.
P305+P351+P338:	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P273:	Avoid release to the environment.
P260:	Do not breathe dust/fume/gas/mist/vapours/spray.
P280:	Wear protective gloves/protective clothing/eye protection/face protection.
P310:	Immediately call a POISON CENTER or doctor/physician.
P405:	Store locked up.

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

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

Product characteristic of the RTP® Mycobacteria Kit

Starting material	Yield	Time	Ratio
Mycobacterial DNA from max. 200 µl Sputum Sample Mycobacterial DNA from Bronchalveolar Lavage Sample Mycobacterial DNA from max. up to 5 ml Sputum Sample Mycobacterial DNA from tissue biopsies (e.g. lymph notes)	depends on the starting material	60-75 min (incl. lysis time)	$A_{260} : A_{280}$ 1.7 – 2.0

The **RTP® Mycobacteria DNA Mini Kit** allows rapid and efficient isolation of high quality genomic DNA from Mycobacteria included in different kinds of starting materials.

The kit uses the patented RTP® technology, whose special feature is the utilization of an **Extraction Tube L** containing pre-formulated solid lysis reagent, lytic enzyme, Proteinase K, carrier nucleic acid and a precisely calibrated amount of an internal DNA Extraction Control.

The internal control is a convenient tool for the assessment and monitoring of extraction efficiency and PCR amplification as well, for the quality of the purified nucleic acid , and for the exclusion of false negative results.)*

Using the **RTP® Mycobacteria Kit,** all types of samples are transferred into the **Extraction Tubes** together with a specially designed **Resuspension Buffer R**. The prefilled buffer and enzymes lyse the samples, stabilize nucleic acid and enhance the selective DNA adsorption to the membrane. The membranes are efficiently washed before the nucleic acid is eluted. In addition to the rigorous lysis procedure, simple pre-treating steps have been introduced, ideally for purification of genomic DNA of different sources. High extraction efficiency and detection sensitivities will be realized. No phenol chloroform extraction is required. The “hands-on time“ necessary for the whole procedure is reduced to a minimum. All kit components can be stored at room temperature.

The procedures require minimal interaction by the user, allowing safe handling of potentially infectious samples.

Due to the high purity, the isolated total DNA is ready to use for a broad panel of downstream applications (see below) or can be stored at –80°C for subsequent use.

- PCR*
- Real-time PCR (quantitative RT-PCR, like TaqMan und LightCycler technology)
- RE Digestion
- RFLP-Analysis
- Sequencing

To purify high chromosomal bacterial DNA in 96 format STRATEC Molecular offers the **Invisorb® Universal Bacterial HTS 96 Kit** for use in a centrifuge, on a vacuum manifold and on common laboratory automated workstations. Furthermore STRATEC Molecular offers the **InviMag® Bacteria DNA Mini Kit KFmL and KF96** for DNA isolation using magnetic beads.

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

*) The PCR method is covered by U.S. Patents 4,683,195 and 4,683,202 owned by Hoffmann-LaRoche Inc. The purchase of the **RTP® Bacteria DNA Mini Kit** cannot be construed as an authorization or implicit license to practice PCR under any patents held by Hoffmann-LaRoche Inc.

Principle and procedure

The **RTP® Mycobacteria Kit** procedure comprises following steps:

- Pre Extraction Processing
- lysis at different temperatures
- binding the nucleic acids in the lysate to the membrane of a RTA Spin Filter
- washing of the membrane and elimination of contaminants and ethanol
- elution of nucleic acid

This manual contains 4 protocols.

Sampling and storage of starting material:

Bacterial cultures
Bacterial cultures grow in the presence of a selective agent such as an antibiotic. The yield and quality of DNA may depend on factors such host strain, inoculation, antibiotic, and type of culture medium. The bacteria will be pelleted after cultivation. Best results are obtained with fresh material or material that has been immediately frozen and stored at –20°C or –80°C. Repeated freezing and thawing of stored samples should be avoided, since this leads to reduced DNA size.

Tissue/ Biopsy Material
Best results are obtained with fresh material or material that has been immediately frozen and stored at –20°C or –80°C. Repeated freezing and thawing of stored samples should be avoided, since this leads to reduced DNA size. Use of poor quality starting material also leads to reduced length and yield of purified DNA. The amount of purified DNA from max. 10 mg tissue sample depends on the nature of starting material.

Sample preparation

Pre Extraction Processing
For sputum sample a pretreatment with **NAC Buffer** is necessary
The sample will be incubate and centrifuged to get a bacteria pellet. This pellet will be resuspend with **Resuspension Buffer R**.

Lysis
Samples are lysed under anti-chaotropic conditions at different, elevated temperature and continuously shaking. Lysis is performed in the presence of **Lysozym** to break the cell wall of the bacteria, a **Lysis Buffer** and **Proteinase K** to digest the proteins. All is provided prefilled in the **Extraction Tube L.** Unlysed sample parts should be removed before the binding step.

Binding genomic DNA
By adding **Binding Solution**to the lysate, optimal binding condition will be adjusted. Each lysate is then applied to a RTA Spin filter and genomic DNA is adsorbed onto membrane as the lysate is drawn through by centrifugal force.

Removing residual contaminants
Contaminants are efficiently washed away using **Wash Buffer's**, while the bacterial, genomic DNA remains bound to the membrane.

Elution of pure genomic DNA
Genomic DNA is eluted from the spin filter using 80 - 100 µl prewarmed **Elution Buffer** or water. Eluting twice each with 80 µl leads to little increase of DNA yield. Usage small elution volumes may raise DNA concentration. Elution volumes should be at least 50 µl. The eluted DNA is ready for use in different downstream applications.

Yield and quality of genomic DNA

The amount of purified DNA using the **RTP® Mycobacteria Kit** procedure, depends on the sample type and the number of mycobacteria cells in the sample (depending from the source , e.g. patients age and health situation, transport conditions, storage, and age of the sample).

For most samples, a single elution with 100 µl **Elution Buffer** is sufficient

Yields of bacterial DNA isolated from biological samples can be small and therefore difficult to determine photometrical.
The kit is suitable for downstream analysis with NAT techniques, for examples qPCR, RT qPCR, LAMP, LCR. Diagnostic assays should be performed according to the manufacturer's instructions.

Quantitative RT-PCR is recommended for determination of mycobacterial DNA yield.

Important notes

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.

- Always change pipet tips between liquid transfers. 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.
- To minimize the risk of infections from potentially infectious material, we recommend working under laminar air-flow until the samples are lysed.
- This kit should only be used by trained personnel.

Internal control (IC)/ Extraction control

Internal Controls (IC) from the PCR assay provider can be used as extraction controls if the fragments are longer than 100 bp. In this case they have to be added after finalization of the lysis step.

Attention: don´t add directly these Internal Controls to the sample!

Preparing reagents and buffers

1. Adjust the thermomixer to 37°C.
2. Warm up the needed amount of **Elution Buffer** to 68°C, (80 - 100 µl **Elution Buffer** are needed per sample).
3. Heat heating blocks (e.g. thermomixer) to 68°C and 95 °C.
4. Label the needed amount of 2.0 ml RTA Spin Filter Sets.
5. Label the needed amount of 1.5 ml Receiver Tubes add the needed amount of ethanol to the **Wash Buffer I** and **II** (see Kit Contents, page 3).

5 bacterial DNA extractions:
Binding Solution, Wash Buffer I and **II** are ready to use! Dilute the **Proteinase K** by addition of 250 µl ddH_20, mix thoroughly and store like described below
50 bacterial DNA-extractions:
Fill 30 ml 99.7% **Isopropanol** (molecular biologic grade) into the empty bottle Add 30 ml of 96-100% ethanol to the bottle **Wash Buffer I**, mix thoroughly and keep the bottle always firmly closed! Add 42 ml of 96-100% ethanol to the bottle **Wash Buffer II**, mix thoroughly and keep the bottle always firmly closed! Dilute the **Proteinase K** by addition of 1 ml ddH_20, mix thoroughly and store like described below
250 bacterial DRNA-extractions:
Fill 120 m 99.7% **Isopropanol** (molecular biologic grade) into the empty bottle Add 80 ml of 96-100% ethanol to the bottle **Wash Buffer I**, mix thoroughly and keep the bottle always firmly closed! Add 105 ml of 96-100% ethanol to the bottle **Wash Buffer II,** mix thoroughly and keep the bottle always firmly closed! Dilute the Proteinase K by addition of 1 ml ddH_20, mix thoroughly and store like described below

Equipment and reagents to be supplied by user

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDS). (See our webpage : www.stratec.com)

- Microcentrifuge
- Eppendorf Thermomixer (68°, 80°C)
- Measuring cylinder (250 ml)
- Disposable gloves
- Pipet with tips
- Reagents reservoirs for multichannel pipets
- 96 -100 % ethanol
- ddH_2O
- Isopropanol*

The **RTP® Mycobacteria Kit** is validated with 2-Propanol; Rotipuran >99.7%, p.a., ACS, ISO (Order no. 6752) from Carl Roth

*** Possible suppliers for Isopropanol:**

Fa. Carl Roth
2-Propanol
Rotipuran >99.7%, p.a., ACS, ISO
Ordering No. 6752

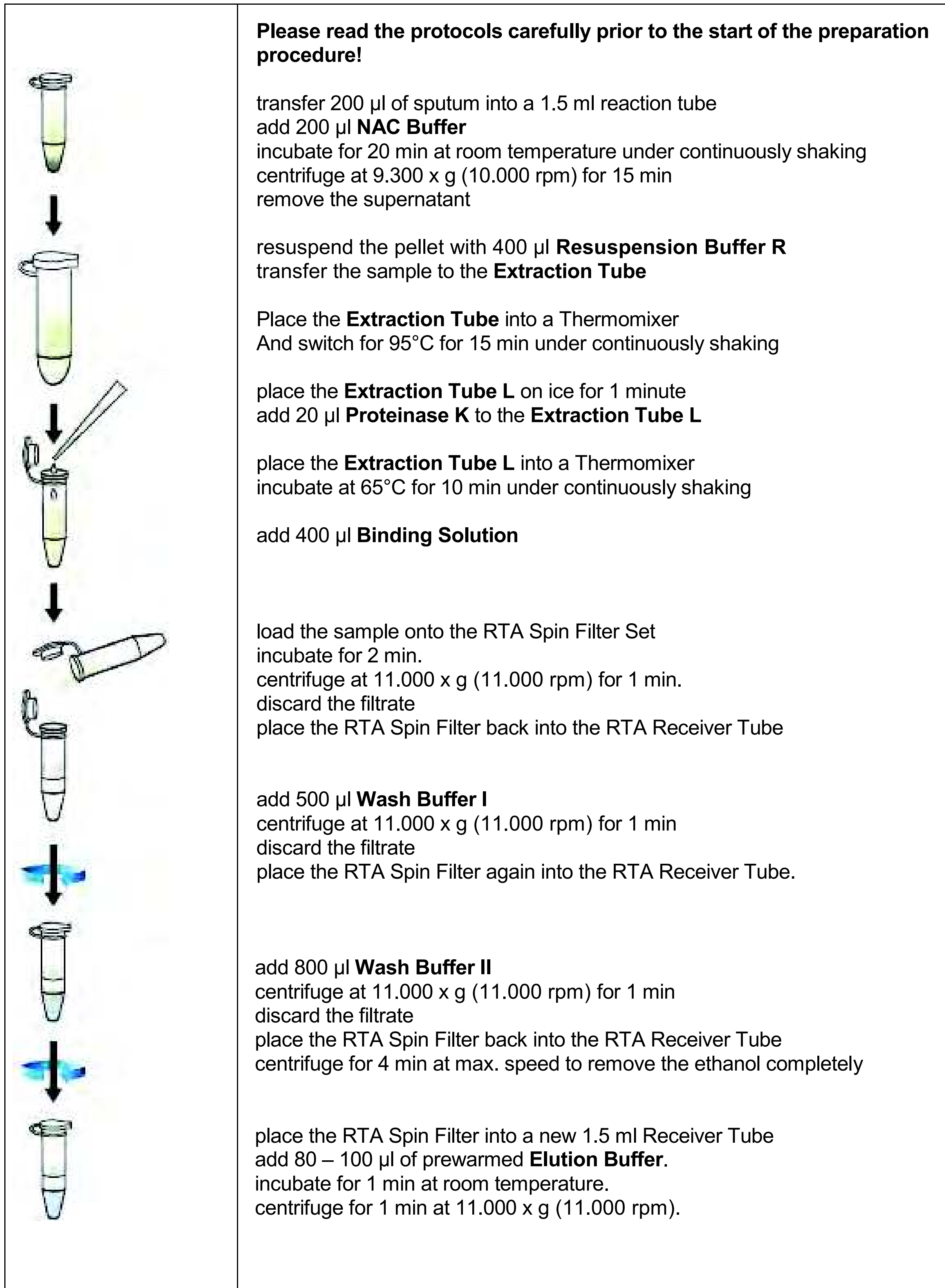
Fa. Applichem
2-Propanol für die Molekularbiologie
Ordering No. A3928

Fa. Sigma
2-Propanol
Ordering No. 59304-1L-F

Important indication

Measurement of the DNA directly after isolation by intercalating fluorescent dyes may lead too low results. This is caused by the nature of the isolated DNA, due the denaturing step (95°C) during lysis DNA is partially single stranded. So fluorophores using double stranded DNA will not work correctly.

Scheme of the RTP® Mycobacteria Kit



Please read the protocols carefully prior to the start of the preparation procedure!

transfer 200 µl of sputum into a 1.5 ml reaction tube
add 200 µl **NAC Buffer**
incubate for 20 min at room temperature under continuously shaking
centrifuge at 9.300 x g (10.000 rpm) for 15 min
remove the supernatant

resuspend the pellet with 400 µl **Resuspension Buffer R**
transfer the sample to the **Extraction Tube**

Place the **Extraction Tube** into a Thermomixer
And switch for 95°C for 15 min under continuously shaking

place the **Extraction Tube L** on ice for 1 minute
add 20 µl **Proteinase K** to the **Extraction Tube L**

place the **Extraction Tube L** into a Thermomixer
incubate at 65°C for 10 min under continuously shaking

add 400 µl **Binding Solution**

load the sample onto the RTA Spin Filter Set
incubate for 2 min.
centrifuge at 11.000 x g (11.000 rpm) for 1 min.
discard the filtrate
place the RTA Spin Filter back into the RTA Receiver Tube

add 500 µl **Wash Buffer I**
centrifuge at 11.000 x g (11.000 rpm) for 1 min
discard the filtrate
place the RTA Spin Filter again into the RTA Receiver Tube.

add 800 µl **Wash Buffer II**
centrifuge at 11.000 x g (11.000 rpm) for 1 min
discard the filtrate
place the RTA Spin Filter back into the RTA Receiver Tube
centrifuge for 4 min at max. speed to remove the ethanol completely

place the RTA Spin Filter into a new 1.5 ml Receiver Tube
add 80 – 100 µl of prewarmed **Elution Buffer**.
incubate for 1 min at room temperature.
centrifuge for 1 min at 11.000 x g (11.000 rpm).

Instructions

The following notes are valid for all protocols:

Note: *The centrifugation steps were made with the **Centrifuge 5415 D from Eppendorf.** The indicated **rpm amounts** are referring to this centrifuge.*

Protocol 1: Isolation of mycobacterial DNA from Sputum Sample (200 µl sample volume)

Important: ***Heat heating blocks (e.g. Thermomixer) to 65°C; prewarm the needed amount of Elution Buffer at 65 8°C***

Be aware of risk of infection when handling material containing MYCOBACTERIA !

1. Pre Extraction Processing
Transfer 200 µl of sputum into a 1.5 ml reaction tube and add 200 µl of NAC Buffer. Vortex shortly and incubate the sample at room temperature under continuously shaking for 20 minutes. Centrifuge the sample at 9.300 x g (10.000 rpm) for 15 min.Remove the supernatant carefully but completely.
Resuspend the resulting pellet with 400 µl Resuspension Buffer R. Transfer the sample completely into the Extraction Tube L. Vortex the tube shortly.

2. Lysis at 95°C for 15 min in a Thermomixer
Place the Extraction Tube L into a Thermomixer and switch 95°C for 15 min under continuously shaking (continuous shaking increases the lysis efficiency).

3. Incubation on ice for 1 min
Place the Extraction Tube L on ice for 1 minute. Open the Extraction Tube L carefully and add 20 µl Proteinase K.

4. Lysis at 65°C for 10 min in a Thermomixer
Place the Extraction Tube L into a Thermomixer and incubate at 68°C for 10 min under continuously shaking (continuous shaking increases the lysis efficiency).

5. Realization of optimal binding condition
Add 400 µl Binding Solutionto the sample and vortex shortly.

6. DNA Binding on RTA Spin filter surface
Load the sample onto the RTA Spin Filter Set and incubate for 2 min. Centrifuge at 11.000 x g (11.000 rpm) in a standard table centrifuge for 1 min. Discard the filtrate and place the RTA Spin Filter back into the RTA Receiver Tube

7. Washing I
Add 500 µl Wash Buffer I and centrifuge at 11.000 x g (11.000 rpm) for 1 min. Discard the filtrate, place the RTA Spin Filter again into the RTA Receiver Tube.

8. Washing II
Add 800 µl Wash Buffer II and centrifuge at 11.000 x g (11.000 rpm) for 1 min. Discard the filtrate, place the RTA Spin Filter back into the RTA Receiver Tube and finally centrifuge for 4 min at max. speed to remove the ethanol completely.

9. Elution of the DNA
Place the RTA Spin Filter into a new 1.5 ml Receiver Tube and add 80 – 100 µl of prewarmed Elution Buffer. Incubate for 1 min at room temperature. Centrifuge for 1 min at 11.000 x g (11.000 rpm).

Optional: If a white pellet is visible in the eluate, spin the sample again at maximum speed and transfer the supernatant into a clean tube!!

Protocol 2: Isolation of Mycobacterial DNA from Bronchoalveolar Lavage Sample

Important: ***Heat heating blocks (e.g. Thermomixer) to 65°C °C; prewarm the needed amount of Elution Buffer at 65°C***

Be aware of risk of infection when handling material containing MYCOBACTERIA !

1. Pre Extraction Processing
Transfer up to 1 ml of bronchalveolar lavage sample and mix it with up to 1 ml of NAC buffer: (ratio 1:1) and incubate for 20 min at RT. Centrifuge the sample at 9.300 x g (10.000 rpm) for 15 min. Remove the supernatant carefully but completely.
Resuspend the resulting pellet with 400 µl Resuspension Buffer R. Transfer the sample completely into the Extraction Tube L. Vortex the tube shortly.

2. Lysis at 95°C for 15 min in a Thermomixer
Place the Extraction Tube L into a Thermomixer and switch for 95°C for 15 min under continuously shaking (continuous shaking increases the lysis efficiency).

3. Incubation on ice for 1 min
Place the Extraction Tube L on ice for 1 minute. Open the Extraction Tube L carefully and add 20 µl Proteinase K.

4. Lysis at 65°C for 10 min in a Thermomixer
Place the Extraction Tube L into a Thermomixer and incubate at 68°C for 10 min under continuously shaking (continuous shaking increases the lysis efficiency).

5. Realization of optimal binding condition
Add 400 µl Binding Solutionto the sample and vortex shortly.

6. DNA Binding on RTA Spin filter surface
Load the sample onto the RTA Spin Filter Set and incubate for 2 min. Centrifuge at 11.000 x g (11.000 rpm) in a standard table centrifuge for 1 min. Discard the filtrate and place the RTA Spin Filter back into the RTA Receiver Tube

7. Washing I
Add 500 µl Wash Buffer I and centrifuge at 11.000 x g (11.000 rpm) for 1 min. Discard the filtrate, place the RTA Spin Filter again into the RTA Receiver Tube.

8. Washing II
Add 800 µl Wash Buffer II and centrifuge at 11.000 x g (11.000 rpm) for 1 min. Discard the filtrate, place the RTA Spin Filter back into the RTA Receiver Tube and finally centrifuge for 4 min at max. speed to remove the ethanol completely.

9. Elution of the DNA
Place the RTA Spin Filter into a new 1.5 ml Receiver Tube and add 80 – 100 µl of prewarmed Elution Buffer. Incubate for 1 min at room temperature. Centrifuge for 1 min at 11.000 x g (11.000 rpm).

Optional: If a white pellet is visible in the eluate, spin the sample again at maximum speed and transfer the supernatant into a clean tube !!

Protocol 3: Isolation of mycobacterial DNA from Sputum Sample of larger volumes (up to 5 ml)

Important: ***Heat heating blocks (e.g. Thermomixer) to 65°C; prewarm the needed amount of Elution Buffer at 65°C;***

Note: ***Order additional NAC Buffer under catalog no.: NAC Buffer 10332211***

Be aware of risk of infection when handling material containing MYCOBACTERIA !

1. Pre Extraction Processing
Transfer up to 5 ml of sputum sample into a 15 ml reaction tube. Inactivate the sample by incubation at 65 ºC for 30 minutes. Add equal volume of NAC Buffer, vortex shortly and incubate the sample at room temperature for 20 minutes. Centrifuge at 960 x g (3.000 rpm) for 15 minutes. Remove the supernatant carefully but completely.
Resuspend the resulting pellet with 400 µl Resuspension Buffer R. Transfer the sample completely into the Extraction Tube L. Vortex the tube shortly.

2. Lysis at 95°C for 15 min in a Thermomixer
Place the Extraction Tube L into a Thermomixer and switch for 95°C for 15 min under continuously shaking (continuous shaking increases the lysis efficiency).

3. Incubation on ice for 1 min
Place the Extraction Tube L on ice for 1 minute. Open the Extraction Tube L carefully and add 20 µl Proteinase K.

4. Lysis at 65°C for 10 min in a Thermomixer
Place the Extraction Tube L into a Thermomixer and incubate at 68°C for 10 min under continuously shaking (continuous shaking increases the lysis efficiency).

5. Realization of optimal binding condition
Add 400 µl Binding Solutionto the sample and vortex shortly.

6. DNA Binding on RTA Spin filter surface
Load the sample onto the RTA Spin Filter Set and incubate for 2 min. Centrifuge at 11.000 x g (11.000 rpm) in a standard table centrifuge for 1 min. Discard the filtrate and place the RTA Spin Filter back into the RTA Receiver Tube

7. Washing I
Add 500 µl Wash Buffer I and centrifuge at 11.000 x g (11.000 rpm) for 1 min.Discard the filtrate, place the RTA Spin Filter again into the RTA Receiver Tube.

8. Washing II
Add 800 µl Wash Buffer II and centrifuge at 11.000 x g (11.000 rpm) for 1 min. Discard the filtrate, place the RTA Spin Filter back into the RTA Receiver Tube and finally centrifuge for 4 min at max. speed to remove the ethanol completely.

9. Elution of the DNA
Place the RTA Spin Filter into a new 1.5 ml Receiver Tube and add 80 – 100 µl of prewarmed Elution Buffer. Incubate for 1 min at room temperature. Centrifuge for 1 min at 11.000 x g (11.000 rpm).

Optional: If a white pellet is visible in the eluate, spin the sample again at maximum speed and transfer the supernatant into a clean tube !

Protocol 4: Isolation of Mycobacterial DNA from tissue biopsies (e.g. lymph notes)

Important: ***Heat heating blocks (e.g. Thermomixer) to 52°C; 68°C and 95 °C; prewarm the needed amount of Elution Buffer at 68°C***

Be aware of risk of infection when handling material containing MYCOBACTERIA !

1. Pre Extraction Processing
Transfer 1mg up to – max. 10 mg of the biopsy into the Extraction Tube L. Add 400 µl of Resuspension Buffer R. Close the cap and vortex shortly. Place the Extraction Tube L into a Thermomixer and incubate under continuously shaking for 30 – 60 min minutes at 52°C. Lysis time can be increased if the lysis is not completely.

2. Lysis at 95°C for 15 min in a Thermomixer
Place the Extraction Tube L into a Thermomixer and switch for 95°C for 15 min under continuously shaking (continuous shaking increases the lysis efficiency).

3. Incubation on ice for 1 min
Place the Extraction Tube L on ice for 1 minute. Open the Extraction Tube L carefully and add 20 µl Proteinase K.

4. Lysis at 65°C for 10 min in a Thermomixer
Place the Extraction Tube L into a Thermomixer and incubate at 68°C for 10 min under continuously shaking (continuous shaking increases the lysis efficiency).

5. Realisation of optimal binding condition
Add 400 µl Binding Solutionto the sample and vortex shortly.

6. DNA Binding on RTA Spin filter surface
Load the sample onto the RTA Spin Filter Set and incubate for 2 min. Centrifuge at 11.000 x g (11.000 rpm) in a standard table centrifuge for 1 min. Discard the filtrate and place the RTA Spin Filter back into the RTA Receiver Tube

7. Washing I
Add 500 µl Wash Buffer I and centrifuge at 11.000 x g (11.000 rpm) for 1 min. Discard the filtrate, place the RTA Spin Filter again into the RTA Receiver Tube.

8. Washing II
Add 800 µl Wash Buffer II and centrifuge at 11.000 x g (11.000 rpm) for 1 min. Discard the filtrate, place the RTA Spin Filter back into the RTA Receiver Tube and finally centrifuge for 4 min at max. speed to remove the ethanol completely.

9. Elution of the DNA
Place the RTA Spin Filter into a new 1.5 ml Receiver Tube and add 80 – 100 µl of prewarmed Elution Buffer. Incubate for 1 min at room temperature. Centrifuge for 1 min at 11.000 x g (11.000 rpm).

Optional: If a white pellet is visible in the eluate, spin the sample again at maximum speed and transfer the supernatant into a clean tube!

Troubleshooting

Problem	Probable cause	Comments and suggestions
clogged Spin-Filter	insufficient lysis and/or too much starting material	increase lysis time increase centrifugation speed reduce amount of starting material
low amount of extracted DNA	insufficient lysis	increase lysis time at 95°C. don’t forget the addition of **Proteinase K** !
	incomplete elution	prolong the incubation time with **Elution Buffer** to 5-10 min or repeat elution step once again. take higher volume of **Elution Buffer**.
	insufficient mixing of the sample with Isopropanol	mix sample with **Binding Solution**completely by pipetting or by vortexing prior to transfer the sample onto the RTA Spin Filter.
low concentration of extracted DNA	too much Elution Buffer	elute the DNA twice with lower volume of **Elution Buffer**
	incorrect storage of starting material	ensure that the storage of starting material was correctly avoid thawing of the material
no Amplification Results	incorrect storage of starting material	ensure that the starting material is fresh or stored under appropriate conditions (for long time storage at – 20°C)! avoid repeating thawing and freezing of the material.
	old material	old material often contains degraded DNA.
	ethanol carryover during elution	increase time for removing of ethanol.
	salt carryover during elution	ensure that Wash Buffer is at room temperature. check up **Wash Buffer** for salt precipitates. If there are any precipitates, solve these precipitates by warming carefully.
RNA contaminations of extracted DNA.		start **RNAse A** digestion (not included in the kit)

Appendix

General notes on handling DNA

Nature of DNA

The length and delicate physical nature of DNA require 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 it will function well in various downstream applications. Damaged DNA could perform poorly in applications such as genomic Southern blotting, long-template PCR, and construction of cosmid libraries.

Handling fresh and stored material before the extraction of DNA

For the isolation of genomic DNA from cells or tissues, use either fresh samples or samples that have been quickly frozen in liquid nitrogen and stored at -70°C. This procedure minimizes degradation of crude DNA by limiting the activity of endogenous nucleases.

Storage of DNA

Store genomic DNA at +2 to +8°C. Storing genomic DNA at -15 to -25°C can cause shearing of DNA, particularly if the DNA is exposed to repeated freeze-thaw cycles. Plasmid DNA and other small circular DNA's can be stored at +2 to +8°C or at -15 to -25°C.

Handling of DNA

Avoid vigorous pipetting. Pipetting 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 pipetting genomic DNA.

Ordering information

Product	Package size	Catalogue No.
RTP® Mycobacteria Kit	5 extractions	1033220100
RTP® Mycobacteria Kit	50 extractions	1033220200
RTP® Mycobacteria Kit	250 extractions	1033220300

Single components

Extraction Tube L	10 tubes	1033221500
NAC Buffer	15 ml	1033221100
Resuspension Buffer R	30 ml	1033222200
Wash Buffer I	30ml	1033223500
Wash Buffer II	18 ml	1033223600
Elution Buffer	15 ml	1033224000

Related Products

RTP® Bacteria DNA Mini Kit	5 extractions	1033200100
RTP® Bacteria DNA Mini Kit	50 extractions	1033200200
RTP® Bacteria DNA Mini Kit	250 extractions	1033200300
InviMag® Bacteria DNA Mini Kit/ KFmL	15 purifications	2433150100
InviMag® Bacteria DNA Mini Kit/ KFmL	75 purifications	2433150200
InviMag® Bacteria DNA Mini Kit/ KF96	1 x 96 purifications	7433300100
InviMag® Bacteria DNA Mini Kit/ KF96	5 x 96 purifications	7433300200
Invisorb Universal Bacteria HTS 96 Kit/X	2 x 96 purifications	7133310200
Invisorb Universal Bacteria HTS 96 Kit/X	4 x 96 purifications	7133310300
Invisorb Universal Bacteria HTS 96 Kit/X	24 x 96 purifications	7133310400

Possible suppliers for Isopropanol:

Fa. Carl Roth
2-Propanol
Rotipuran >99.7%, p.a., ACS, ISO
Ordering No. 6752

Fa. Applichem
2-Propanol für die Molekularbiologie
Ordering No. A3928

Fa. Sigma
2-Propanol
Ordering No. 59304-1L-F

Possible suppliers for Centrifuges:

Eppendorf AG
22331 Hamburg, Germany
Phone: +49 (0) 40 53801 0
Fax: +49 (0) 40 53801 556
E-Mail: eppendorf@eppendorf.com
Internet: www.eppendorf.com

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