Instruction InviMag® Stool DNA Kit/ KFmL

The **InviMag® Stool DNA Kit/ KFmL** combines the advantages of the innovative Invisorb® technology with easy handling of magnetic particles for a very efficient and reliable isolation of nucleic acids with a high purity.

The DNA-binding magnetic particles are characterized by a high surface area, a uniform size distribution, and a good suspension stability and are therefore highly suitable for high throughput processing.

The **InviMag® Stool DNA Kit/ KFmL** for isolation and efficient purification of high purity DNA from pathogenic microorganisms, as well as for isolation of DNA from the host organism. Furthermore, it is possible to extract nucleic acids from food and feed residues of plant or animal origin from max. 200 mg of fresh or frozen stool sample. The **InviMag® Stool DNA Kit/ KFmL** has been designed for an optimal use on the KingFisher® mL workstation from Thermo Scientific. The interplay of the DNA extraction and purification chemistry provided by the **InviMag® Stool DNA Kit/ KFmL** with the KingFisher machine was intensely tested and validated.

Due to the high purity, the isolated DNA is ready to use for *in vitro* diagnostic analysis and for a broad panel of downstream applications or can be stored at – 20 ℃ for subsequent use.

The kit is neither validated for the isolation of genomic DNA from cultured or isolated cells, from tissue, swabs, dried blood stains, or cell free body fluids, like synovial fluid and urine or the purification of RNA. The application of the kits for isolation and purification of viral DNA has not been evaluated.

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

Contents

Kit contents of the InviMag® Stool DNA Kit/ KFmL

Store the **MAP Solution A** at 4℃! Store lyophilized **Proteinase K** at 4 - 8 ℃!
Store diluted **Proteinase K** at –20℃, but repeated freezing and thawing will r educed the activity dramatically, dividing the **Proteinase K** into aliquots and storage at –20℃ is recommended.
Store all other kit components at room temperature

	15 extraction	**75 extraction**
Catalogue Number	2438110100	2438110200
2,0 ml Receiver Tubes	15	5 x 15
InviAdsorb	15	5 x 15
Zirkonia Beads II	1 vial	2 vials
1.5 ml Receiver Tubes	2 x 15	3 x 50
Lysis Buffer P	30 ml	100 ml
Proteinase K	0.5 ml working solution	2 ml working solution
MAP Solution A	0.5 ml	2 x 1 ml
Binding Buffer P	8 ml	30 ml
Wash Buffer I	15 ml ready to use	2 x 30 ml final volume 2 x 60 ml
Wash Buffer II	2 x 15 ml ready to use	45 ml final volume 150 ml
Elution Buffer D	2 x 2 ml	30 ml
KingFisher mL Tip Combs	3	15
KingFisher mL Tube Strips	15	75
Manual	1	1
Initial steps	**Wash Buffer I** is ready to use **Wash Buffer II** is ready to use Dilute **Proteinase K** by addition of 0.5 ml of ddH_2O, mix thoroughly and store like described!	Add 30 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 **Proteinase K** by addition of 2 ml of ddH_2O, mix thoroughly and store like described!

Kit contents of the InviMag® Stool DNA Kit/ KFmL / wp

Store the **MAP Solution A** at 4℃! Store lyophilized **Proteinase K** at 4 - 8 ℃!
Store diluted **Proteinase K** at –20℃, but repeated freezing and thawing will r educed the activity dramatically, dividing the **Proteinase K** into aliquots and storage at –20℃ is recommended.
Store all other kit components at room temperature

	15 extraction	**75 extraction**
Catalogue Number	2438110150	2438110250
2,0 ml Receiver Tubes	15	5 x 15
InviAdsorb	15	5 x 15
Zirkonia Beads II	1 vial	2 vials
1.5 ml Receiver Tubes	2 x 15	3 x 50
Lysis Buffer P	30 ml	100 ml
Proteinase K	0.5 ml working solution	2 ml working solution
MAP Solution A	0.5 ml	2 x 1 ml
Binding Buffer P	8 ml	30 ml
Wash Buffer I	15 ml ready to use	2 x 30 ml final volume 2 x 60 ml
Wash Buffer II	2 x 15 ml ready to use	45 ml final volume 150 ml
Elution Buffer D	2 x 2 ml	30 ml
Manual	1	1
Initial steps	**Wash Buffer I** is ready to use **Wash Buffer II** is ready to use Dilute **Proteinase K** by addition of 0.5 ml of ddH_2O, mix thoroughly and store like described!	Add 30 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 **Proteinase K** by addition of 2 ml of ddH_2O, mix thoroughly and store like described!
Plastic to be supplied by user (see order information)		
KingFisher mL Tip Combs	3	15
KingFisher mL Tube Strips	15	75

Symbols

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® Stool DNA Kit/ KFmL,** except **MAP Solution A,** are stable for at least 12 months. **MAP Solution A** is stable for at least 6 months.

All buffers and kit contents of the **InviMag® Stool DNA Kit/ KFmL**, except **Proteinase K** and **MAP Solution A,** should be stored at room temperature (RT)

Proteinase K: Lyophilized Proteinase K should be stored at 2-8 °C. Dissolved Proteinase K must be stored at –20 °C. Dividing the Proteinase K into aliquots and storage at –20 °C is recommended.

MAP Solution A: should be stored at 4 °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 room temperature (up to 30 °C).

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

Quality control

STRATEC Molecular guarantees the correct function of the **InviMag® Stool DNA Kit/ KFmL**, for applications as described in the manual. In accordance with STRATEC Molecular's certified QM-System each component of the **InviMag® Stool DNA Kit/ KFmL**, was tested against predetermined specifications to ensure consistent product quality.

If you have any questions or problems regarding any aspects of **InviMag® Stool DNA Kit/ KFmL**, or other STRATEC Molecular products, please do not hesitate to contact us.

For technical support or further information please contact: +49 (0) 30 9489 2907

or your local distributor (see page 22)

Intended use

The **InviMag® Stool DNA Kit/ KFmL** has been designed for fast and efficient purification of genomic and microbial DNA from fresh and frozen human or animal stool samples or from other sample types with high concentrations of PCR inhibiting components.

Stool samples typically contain many compounds that can degrade DNA and inhibit downstream enzymatic reactions. The **InviMag® Stool DNA Kit/ KFmL,** optimized the essential washing conditions to remove all potent inhibitors very efficient.

The **InviMag®** technology combines the advantages of the innovative **Invisorb®** technology for isolation of genomic DNA (without chaotropic buffer components) with the easy handling of magnetic particles for a highly efficient and reliable purification of highly purified genomic DNA. The **InviMag® Kits/ KFmL** have been designed for an optimal use on KingFisher mL workstations from Thermo Electron Cooperation.

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 diagnostic results generated 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.

Product use limitation

The kit is neither validated for the isolation of genomic DNA from cultured or isolated cells, from tissue, swabs, dried blood stains, or cell free body fluids, like synovial fluid and urine or the purification of RNA. The application of the kit for isolation and purification of viral DNA has not been evaluated.

The included chemicals 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 STRATEC Molecular kits for any particular use, since the performance characteristics of our kits have not been validated for any specific application. STRATEC Molecular 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.

The chemicals and plastic parts are for laboratory use only, they must be stored in the laboratory and must not used for purposes other 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 and avoid skin contact. Heed 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.invitek.de for each STRATEC Molecular 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.

STRATEC Molecular has not tested the liquid waste generated by the **InviMag® Stool DNA Kit/ KFmL** 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 must be considered infectious and be handled and discarded according to local safety regulation.

Below is listed European Community risk and safety phrases for the components of the **InviMag® Stool DNA Kit/ KFmL** to which they apply.

Lysis Buffer P

danger

H319 P305-351-338

Binding Buffer P

danger

H225-319-336 P210-233-305-351-338

Proteinase K

danger

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

Wash Buffer I

warning

H302-312-332-412 EUH032 P273

H315:	Causes skin irritation.
H319:	Causes serious eye irritation.
H334:	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335:	May cause respiratory irritation.
H225:	Highly flammable liquid and vapour.
H336:	May cause drowsiness or dizziness.
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.
P280:	Wear protective gloves/protective clothing/eye protection/face protection.
P305+P351+P338:	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310:	Immediately call a POISON CENTER or doctor/physician.
P405:	Store locked up.
P210:	Keep away from heat/sparks/open flames/hot surfaces. — No smoking.
P233:	Keep container tightly closed.
P273:	Avoid release to the environment.

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® Stool DNA Kit Kit/ KFmL

Starting material	Yield	Time	Ratio
200 - 400 mg fecal sample	up to 50 µg (depends on starting material)	about 45 min (incl. lysis time)	A_{260} : A_{280} 1.4 – 1.8

The **InviMag® Stool DNA Kit/ KFmL** allows a semi automated rapid and efficient isolation of high quality DNA from up to 200 – 400 mg of fresh or frozen human and animal stool sample using magnetic beads.The process is a standardized procedure, reducing mistakes in analysis.

The isolation protocol as well as all buffers are optimized to provide high yield and purity of the isolated DNA. The “hands-on time“ necessary for the whole procedure is reduced to minimum.

Stool samples typically contain many compounds that can degrade DNA and inhibit downstream enzymatic reactions. During the transport of stool samples from one place to the other at RT, DNA will be massively digested, and the pathogen load is changing during transportation. To prevent all these problems STRATEC Molecular offers transport containers with Stool DNA Stabilizer. The Stool Collection Tubes contains 8 ml of Stool DNA Stabilizer, a buffer formulation, which prevent DNA digestion, freeze the microorganism load, enables the prelyses of the sample and stabilization of the DNA for at least 3 days at ambient temperature. Beside saving and stabilization of the traces of human DNA, the Stool DNA Stabilizer is also very successful if bacterial pathogens should be detected, which are difficult to lyse because of the structure of their cell walls. This Stool Collection Tubes with Stool DNA Stabilizer can be ordered separately (order no: 1038111200).

A rigorous prelysis steps with optimized prelysis buffer under high temperatures, is followed by an preincubation of the sample with **InviAdsorb** to remove PCR inhibitors. Undissolved particles and PCR inhibitors bound to InviAdsorb will be removed by a centrifugation step. The following Proteinase K digestion ensures high yields. Stool contains a range of DNA e.g. host DNA from colon epithelial cells, parasite DNA, bacterial DNA, DNA from food or DNA from gastrointestinal pathogens The choose of different lysis conditions allow the enrichment or a reduction of the content of bacterial DNA in the total DNA in favor of human DNA. The DNA binds to the surface of the magnetic particles. The **InviMag® Stool DNA Kit/ KFmL** optimized the essential washing conditions to remove all potent inhibitors very efficient.

All impurities are very efficiently removed in wash steps and the purified DNA is eluted directly in a low-salt buffer. No phenol/chloroform extraction or ethanol precipitation is necessary. The kit provides reproducible recovery rates of highly purified DNA, ready to use in any downstream application. The isolated DNA can be stored at -20℃ for later use.

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℃ for subsequent use.

- PCR Applications*
- Hybridization
- Genetic typing
- Pathogen typing
- Mutation analysis
- Paternity analysis

Traditional time-killing procedures can be replaced using the **InviMag® Stool DNA Kit/ KFmL.**

* 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 InviMag® **Stool DNA Kit/ KFmL** cannot be construed as an authorization or implicit licence to practice PCR under any patents held by Hoffmann-LaRoche Inc

Sampling and storage of starting material

The collected fresh stool sample can be stored at ambient temperature for at least 1-2 hours at RT, but the high content of DNases realize quickly a DNA digestion and degradation. The sample should be quickly added to the lysis buffer or can be stored frozen at – 20℃ for weeks.

The storage of fresh samples under Stool DNA Stabilizer allow a storage at RT for about 3 days. The storage of fresh samples under Stool DNA Stabilizer will lead to less degraded DNA, a better yield of bacterial pathogens with difficult to lyse cell walls. Storage time has no influence on the quality or the amount of host cell DNA.

The collected sample under Stool DNA Stabilizer can also be used immediately after collection for the isolation of DNA.

The collected sample can be refrigerated at –20℃ immediately after collection or after storage at ambient temperature for a later use (for example for a second DNA isolation).

Principle and procedure

The **InviMag® Stool DNA Kit/ KFmL** procedure comprises following steps:

- stabilization and lyses of sample
- removal of PCR Inhibitors
- protein digestion
- binding the nucleic acids to magnetic particles
- washing of the beads and elimination of contaminants and ethanol
- elution of the nucleic acids

After homogenization of the sample in the Lysis Buffer or Stool DNA Stabilizer which inactivate DNAses, the human and bacterial cell wall will be lysed more or less (depending from the temperature profile), and mixed with **InviAdsorb** the most PCR, followed by a Protein digestion. After lysis the DNA binds to the magnetic beads, contaminations and enzyme inhibitors are efficiently removed during the following three wash steps and highly purified DNA is eluted in Elution Buffer or water.

This manual contains 2 protocols.

Procedure

Lysis

Stool samples are lysed in 1,5 ml tubes outside of the KingFisher ml platform in the presence of **Proteinase K and Lysis Buffer P** under denaturing conditions at elevated temperatures. Human cells lyse efficiently at RT, bacterial cells and those of other pathogens in the stool sample are efficiently lysed by incubation at 95℃. This is recommended for detection of cells that are difficult to lyse (e.g. gram positive bacteria).

Note: *The total DNA concentration in the lysate will be increased 3-5 fold by lysis at 95℃ and the ratio of nonhuman to human DNA will increase.*

Removal of PCR inhibitors

After lysis DNA damaging substances and PCR inhibitors present in the feces are adsorbed efficiently to the **InviAdsorb** matrix. **InviAdsorb** is provided very convenient in safe look tubes and the lysate must only be mixed with the matrix. The bound contaminations and cell debris are pelleted then by centrifugation and the supernatant contains the precleaned DNA.

Binding of total DNA
After adding **Binding Buffer P** and **MAP Solution A** to the supernatant in the Tube A, the DNA is bound to the surface of the magnetic beads.

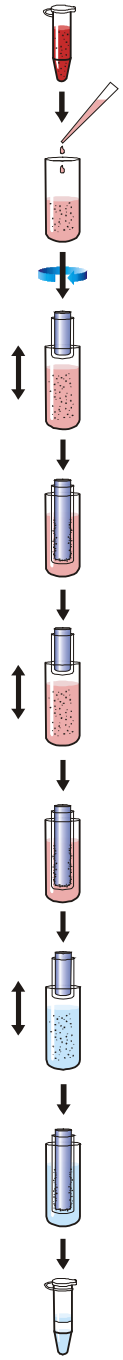
Removing residual contaminants
Contaminants are efficiently washed away using **Wash Buffer I** and **II**, while the DNA remains bound to the magnetic beads.

Elution
The nucleic acids are eluted in low salt buffer from the membrane using 100 - 200 µl **Elution Buffer D**. The eluted nucleic acids are ready for use in different subsequent tests.

Yield and quality of genomic DNA

The amount of purified DNA in the **InviMag® Stool DNA Kit/ KFmL** procedure from feaces, depends on the healthy status of the donor, the bacteria content, sample source, transport, storage, and age. Yield and quality of isolated genomic DNA is suitable for any molecular-diagnostic detection system. The diagnostic tests should be performed according to manufacturers' specifications.

Scheme of the InviMag® Stool DNA Kit / KFmL



Please read protocols prior the start of the preparation carefully

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Transfer 200 mg of the sample into a 2 ml /10 ml reaction tube (not provided) and add 1.2 ml **Lysis Buffer P**, vortex.

For enrichment of bacterial DNA incubate 10 min at 95℃ on a thermomixer under shaking, spin down for 1 min.

Transfer the supernatant to the **InviAdsorb** Tube and mix it by vortex.

Incubate 1 min at RT and spin down for 3 min.

Add 25 µl **Proteinase K** and 400µl of the supernatant, in 1.5 ml Reaction Tube and mix it.

Incubate for 10 min at 70 ℃ while continuously shaking on a thermomixer.

During lysis time prefill all KingFisher Tube strip with needed buffer and the appropriate buffer volume

Tube A: 300 µl **Binding Buffer P** and 20 µl **MAP**

Tube B: 800 µl **Wash Buffer I**

Tube C: 800 µl **Wash Buffer II**

Tube D: 800 µl **Wash Buffer II**

Tube E: 200 µl **Elution Buffer D** (prewarmed)

Addition of 400 µl of the lysed sample to **Invimag® beads** in the 5-tube strip of Tube A

DNA binding to magnetic particle

Magnet bead separation

3 x Washing of the particle fixed DNA

3 x Magnet bead separation

Ethanol Removal

Elution of genomic DNA

Removal of beads

pure total DNA

Important points before starting a protocol

After receiving the kit, check the kit components for damage. If buffer bottles are damaged, contact the STRATEC Molecular Technical Services or your local distributor. In case of liquid spillage, refer to "Safety information's" (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 kit, 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

Preparing reagents and buffers

15 viral RNA-extractions:
Add 500 µl dd H_2O to the tube **Proteinase K**, mix thoroughly (vortex 5 sec) and store the tube at -20°C! **Wash Buffer I** is ready to use **Wash Buffer II** is ready to use
75 viral RNA-extractions:
Add 2 ml dd H_2O to the tube **Proteinase K**, mix thoroughly (vortex 5 sec) and store the tube at -20°C! Add 30 ml of 96 – 100 % ethanol to the bottle **Wash Buffer I** and 105 ml of 96 – 100 % ethanol to the bottle **Wash Buffer II**, mix thoroughly and always keep the bottle firmly closed!

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 web page : www.invitek.de)

- microcentrifuge
- Eppendorf Thermomixer (for 80°C)
- measuring cylinder (250 ml)
- disposable gloves
- pipet with tips
- 99,8 % ethanol
- ddH_2O

Protocol 1: Isolation of genomic DNA from up to 200 mg stool samples with and without enrichment of bacterial DNA

Please read the instructions carefully and conduct the prepared procedure.

Important Note: *Please note, that the extracted DNA from stool sample is by the majority from bacterial origin !*

1. Sample Lysis

Weigh 200 - 400 mg of stool sample (fresh or frozen) into a **2.0 ml Receiver Tube.**
Add 1.2 ml **Lysis Buffer P** to each stool sample. Vortex vigorously for 1 min.

Important: *If the sample is liquid, pipet 200 - 400 µl into the **2.0 ml Receiver Tube**.*
*Cut the end of the pipet tip to make pipetting easier.**Vortex the sample for 10 s !***

For an enrichment of bacterial DNA :

Incubate the sample for 10 min at 95°C in a thermomixer under continuously shaking at 900 rpm. Centrifuge the sample at 16.300 x g (14.000 rpm) for 1 min to pellet solid stool particles.

For an enrichment of host DNA, don' t perform this temperature step.

Incubate the sample for 10 min at RT under continuously shaking at 900 rpm. Centrifuge the sample at 16.300 x g (14.000 rpm) for 1 min to pellet solid stool particles.

2. Removal of PCR Inhibitiors

Transfer the supernatant into a **InviAdsorb -Tube** and vortex vigorously for 15 sec.

Incubate the suspension for 1 min at room temperature. Centrifuge the sample at 16.300 x g (14.000 rpm) for 3 min. Transfer the supernatant completely into a new 1.5 ml Receiver Tube; discard the pellet. Centrifuge the sample at 16.300 x g (14.000 rpm) for 3 min.

3. Protein Digestion

Transfer 25 µl **Proteinase K** into a new 1.5 ml Receiver Tube and pipett 400 µl of the supernatant to the 1.5 ml centrifuge tube containing **Proteinase K**, mix shortly by vortexing and incubate the sample for 10 min at 70°C in a thermomixer under continuously shaking at 900 rpm.
During the sample lysis prefill the tubes of the KingFisher tube strips with the following Buffers respectively.

4. KingFisher mL tube strip setup

During the sample lysis prefill the tubes of the KingFisher tube strips with the following Buffers respectively.

Prewarm the **Elution Buffer D** to 70°C (e.g. transfer the needed volume into a tube and place it at the appropriate temperature into a thermomixer)

Note: ***It is important to mix the bottle with MAP Solution A carefully by vigorously shaking or vortexing***

Tube A: 300 µl **Binding Buffer P** and 20 µl **MAP Solution** A
Tube B: 800 µl **Wash Buffer I**
Tube C: 800 µl **Wash Buffer II**
Tube D: 800 µl **Wash Buffer II**
Tube E: later 200 µl **Elution Buffer D**, **prewarmed to 70°C**

5. Place the KingFisher tip combs into the slots!

6. After lysis transfer 425 µl of the lysed sample into the Tube A of the KingFisher tube strip and place the tube tray into the KingFisher system on the right position.

7. START KingFisher System program "**InviMAG_Stool_KFmL**"

8. If the message occur insert 200 µl Elution Buffer for each sample, if finished press Start The buffer has to be taken directly from the incubator at 70°C.

9. After finishing the KingFisher process transfer the DNA into 1.5 ml Receiver Tubes and store at –20°C

Note: ***If the DNA contains carryover of MAP Solution A, centrifuge at maximum speed for 1 minute and pipet the DNA into a new tube.***

Protocol 2: Isolation of genomic DNA from stabilized stool samples with and without enrichment of bacterial DNA

Please read the instructions carefully and conduct the prepared procedure.

Important Note: ***Please note, that the extracted DNA from stool sample is by the majority from bacterial origin !***

Important Note: ***The Stool Collection Tubes with Stool DNA Stabilizer can be ordered separately (order no: 1038111200).***

1. Collection of the stool sample and stabilization

Note: *The Stool Collection Tubes contain 8 ml of Stool DNA Stabilizer. That is a new developed buffer formulation, which enables the prelyses of the sample and stabilization of the DNA for at least 3 days at ambient temperature. The Stool DNA Stabilizer is very successful even if bacterial pathogens should be detected, which are difficult to lyse cause of the structure of their cell walls.*

1. Open the Stool Collection Tube and collect a spoon (~1 g) of the fresh stool sample.
2. Transfer the spoon with the stool sample back into the Stool Collection Tube and close the tube very tight.
3. Mix the sample for a short time by shaking. That will lead to homogenization of the stool sample.

The collected sample can be refrigerated at –20°C immediately after collection or after storage at ambient temperature for a later use (for example for a second DNA isolation).

2. Lysis

For an enrichment of host DNA

Add 1,4 ml of homogenized stool sample to 2 ml Receiver Tube and incubate for 10 min at 70°C

For an enrichment of bacterial DNA :

Add 1,4 ml of homogenized stool sample to 2 ml Receiver Tube and incubate for 10 min at 70°C vortex the sample and incubate for further 20 min at 95°C.

Attention: *If you want to lyse mycobacteria a temperature shock (short incubation on ice after 10 minutes 95°C lyses time, and then putting back to 95° incubation) improves lyses of these specific bacteria.*

1. Centrifuge at 10.000 x g (10.500 rpm) for 3 min
2. Add the supernatant to **InviAdsorb** Tube, resuspend the complete **InviAdsorb** pellet by vortexing and incubate for 1 min at room temperature
3. Centrifuge at 10.000 x g (10.500 rpm) for 3 min

3. Protein Digestion

1. Transfer 600 µl supernatant of step 3 to a 1.5 ml Receiver Tube and add 25 µl Proteinase K
2. Incubate at 65°C for 30 min under continuous shaking
3. Centrifuge at 10.000 x g for 3 min to remove all **InviAdsorb** from lysate
4. Transfer 625 µl of the supernatant to KingFisher Tube A

4. KingFisher mL tube strip setup

During the sample lysis prefill the tubes of the KingFisher tube strips with the following Buffers respectively.

Prewarm the **Elution Buffer D** to 70°C (e.g. transfer the needed volume into a tu be and place it at the appropriate temperature into a thermomixer)

Note: ***It is important to mix the bottle with MAP Solution A carefully by vigorously shaking or vortexing***

Tube A: 300 µl **Binding Buffer P** and 20 µl **MAP Solution A**
Tube B: 800 µl **Wash Buffer I**
Tube C: 800 µl **Wash Buffer II**
Tube D: 800 µl **Wash Buffer II**
Tube E: later 220 µl **Elution Buffer D**, prewarmed to 70° C

Place the KingFisher tip combs into the slots!

After lysis transfer 625 µl of the lysed sample into the Tube A of the KingFisher tube strip and place also the tube tray into the KingFisher system on the right position.

START KingFisher System program "**InviMAG_Stool_KFmL**"

If the message occur insert 200 µl Elution Buffer for each sample, if finished press Start The buffer has to be taken directly from the incubator at 70°C.

After finishing the KingFisher process transfer the DNA into 1.5 ml Receiver Tubes and store at –20°C

Note: ***If the DNA contains carryover of MAP Solution A, centrifuge at maximum speed for 1 minute and pipet the DNA into a new tube.***

For self programming of the KingFisher mL System

To create the program “InviMAG_Stool_KFmL” please use the following printout for the programming with the KingFisher Software:

[PROTOCOL PROPERTIES]

Name = InviMAG_Stool_KFmL
Protocol template version = 2.6.0
Instrument type = KingFisher mL
Creator = STRATEC Molecular GmbH
Created = 8/8/2006 10:45:38
Description = Validation KFmL protocol (Thermo Electron) for isolation of DNA from Stool samples
Kit = InviMAG Stool DNA KFmL
Plate layouts = Stool DNA

[PLATE LAYOUTS]

Stool DNA

Plate type = KingFisher tubestrip 1000 ul
Plate change message = Press START

A:
- volume = 625, name = Sample
- volume = 300, name = Binding Buffer P
- volume = 20, name = MAP Solution A

B:
- volume = 800, name = Wash Buffer I

C:
- volume = 800, name = Wash Buffer II

D:
- volume = 800, name = Wash Buffer II

E:
- volume = 200, name = Elution Buffer D

[STEPS]

BIND
Step parameters
Name = Bind
Well = A, Stool DNA

Beginning of step:
Premix = Yes

Bind parameters:
Bind time = 5min 0s, speed = Grind mix

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

WASH
Step parameters
Name = Wash_1
Well = B, Stool DNA

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

Wash parameters:
Wash time = 1min 30s, speed = Fast dual mix

End of step:
Collect beads = Yes, count = 2

WASH
Step parameters
Name = Wash_2
Well = C, Stool DNA

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

Wash parameters:
Wash time = 1min 0s, speed = Fast dual mix

End of step:
Collect beads = Yes, count = 2

WASH
Step parameters
Name = Wash_3
Well = D, Stool DNA

Beginning of step:
Release = Yes, time = 0s, speed = Fast
Wash parameters:
Wash time = 30s, speed = Fast dual mix

End of step:

Collect beads = Yes, count = 3

DRY

Step parameters

Name = Dry

Well = D, Stool DNA

Dry time = 5min 0s

Tip position = Outside well

ELUTION

Step parameters

Name = Elution

Well = E, Stool DNA

Beginning of step:

Release = Yes, time = 0s, speed = Fast

Elution parameters:

Elution time = 0s, speed = Medium

Pause parameters:

Pause for manual handling = Yes, message = Load Elution Buffer

Postmix time = 5min 0s, speed = Medium

Remove beads:

Remove beads = Yes, collect count = 3, disposal well = B

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 MAP Solution A	mix **MAP Solution A** thoroughly before pipeting to the KingFisher tube
low concentration of extracted DNA	too much Elution Buffer	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 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°C)! 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

Appendicies

KingFisher Software 2.6.2

KingFisher Software 2.6.2 is used to create protocols for the *KingFisher*, *KingFisher mL* and *KingFisher 96* instruments. Once a protocol has been created, the user can either transfer the protocol into the KingFisher instrument memory or run the protocol directly from the software. Directly run protocols are not stored in the instrument memory.

Note! When creating the protocol using KingFisher 96 and Microtiter Deep Well plates (Thermo Electron) it is essential to use KingFisher software 2.6 or 2.6.2 for protocol development as these software versions include correct adjustments for this plate. It is highly recommended to use Thermo Microtiter Deep Well plates with KingFisher 96 instrument to ensure the best purification result.

Checking the PC requirements

The table below lists the PC requirements for KingFisher Software 2.6.2

PC requirements	
Interface	Serial communication port via an RS-232 full duplex interface
Supported operating systems	Microsoft Windows 2000 Microsoft Windows XP Professional
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˚C for several weeks. For long term storage DNA should be stored at -20˚C, but storing at – 20°C 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 over drying 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 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.

DNA Yield

The amount of purified DNA, depends sample source, transport, storage, and age.

Ordering information

Product	Package size	Order Nr.
InviMag® Stool DNA Mini Kit	10 purifications	1438100900
InviMag® Stool DNA Mini Kit	50 purifications	1438100200
InviMag® Stool DNA Mini Kit	250 purifications	1438100300
InviMag® Stool DNA Mini Kit/ KFmL	1 x 15 purifications	2438110100
InviMag® Stool DNA Mini Kit/ KFmL	1 x 75 purifications	2438110200
InviMag® Stool DNA Mini Kit/ KF96	1 x 96 purifications	7438300100
InviMag® Stool DNA Mini Kit/ KF96	5 x 96 purifications	7438300200

Related products

PSP® Spin Stool DNA Kit	3 extractions	1038100100
PSP® Spin Stool DNA Kit	50 extractions	1038100200
PSP® Spin Stool DNA Kit	250 extractions	1038100300
PSP® Spin Stool DNA *Plus* Kit	3 purifications	1038110100
PSP® Spin Stool DNA *Plus* Kit	50 purifications	1038110200
PSP® Spin Stool DNA *Plus* Kit	250 purifications	1038110300
Stool Collection Tubes with DNA Stabilizer	5 tubes	1038111200
InviTaq Hot Start DNA Polymerase	500 Units	3020110300
InviTaq Hot Start DNA Polymerase	1000 Units	3020110400
2X Red Hot Start PCR Master Mix	100 Units	3020110300
2X Red Hot Start PCR Master Mix	500 Units	3020110400
2X Hot Start QPCR Master Mix	200 Units	3020110300
2X Hot Start QPCR Master Mix	400 Units	3020110400
I-Solution (Amplification Enhancer for DNA from feaces)	3 x 0.5 ml	1038113900