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USER MANUAL

InviMag® Stool DNA Kit/ KF96

for semi-automated purification of total genomic DNA from up to 200 mg stool samples with magnetic beads on the KingFisher™ 96 and KingFisher™ Flex, Thermo Fisher Scientific

REF 7438300200

Invitek Molecular GmbH · D-13125 Berlin

Instruction InviMag® Stool DNA Kit/ KF96

The **InviMag® Stool DNA Kit/ KF96** is the ideal tool using the Invisorb® technology in combination with magnetic beads for rapid and efficient isolation and purification of DNA from max. 200 mg of fresh or frozen stool sample on a Thermo Scientific instrument. The purified DNA is of high quality and can be directly used for downstream analysis like metagenomics analysis.

The kit is neither validated for the isolation of genomic DNA from cultured or isolated cells, from tissues, swabs, dried blood stains or cell free body fluids, like synovial fluid and urine nor for the purification of RNA or any kind of viral nucleic acids.

Table of Content

Kit contents of the InviMag® Stool DNA Kit/ KF96

	5 x 96 extractions
Catalogue No.	7438300200
Lysis Buffer P	3 x 210 ml
Binding Buffer A	2 x 24 ml (final volume 2 x 80 ml)
Zirconia Beads II	14 vials
MAP Solution A	10.5 ml
Proteinase K	for 10 x 1.5 ml working solution
Wash Buffer I	3 x 80 ml (final volume 3 x 160 ml)
Wash Buffer II	5 x 60 ml (final volume 5 x 200 ml)
Elution Buffer	120 ml
InviAdsorb	10 x 50
2.0 ml Deep Well Plate	20
KF 96 Tip Comb for DW magnets	5
200 µl Elution Plate	2 x 5
1.5 ml Receiver Tube	10 x 50
Manual	1
Initial steps	Add 56 ml of 99.7% isopropanol to each **Binding Buffer A** bottle. Mix by intensive shaking for 1 min. Shortly before use mix by inverting several times. Add 80 ml of 96-100% ethanol to each bottle **Wash Buffer I**, mix thoroughly and keep the bottle always firmly closed! Add 140 ml of 96-100% ethanol to each **bottle Wash Buffer II**, mix thoroughly and keep the bottle always firmly closed! Dilute each **Proteinase K** by addition of 1.5 ml of ddH_2O, to each vial, mix thoroughly until completely dissolving

Symbols

 Manufacturer

 Lot number

 Catalogue number

 Expiry date

 Consult operating instructions

 Temperature limitation

 Do not reuse

 Humidity limitation

Attention: *Do not combine components of different kits, unless the lot numbers are identical!*

Storage

All buffers and kit contents of the **InviMag® Stool DNA Kit/ KF96,** except dissolved **Proteinase K** and **MAP Solution A** should be stored at room temperature and are stable for at least 12 months.

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

Before every use, make sure that all components have room temperature. If there are any precipitates within the provided solutions solve these precipitates by warming carefully (up to 30°C).

Proteinase K: Dissolved Proteinase K must be stored at 2 - 8 °C for up to two months. For longer storage –20 °C is recommended, freeze-thaw once only. Therefore, the dissolved Proteinase K is stable as indicated on the kit package.

MAP Solution A: The magnetic beads should be stored at 2-8°C.

Wash Buffers charged with ethanol should be appropriately sealed and stored at room temperature.

Binding Buffer charged with isopropanol should be appropriately sealed and stored at room temperature.

Quality control and product warranty

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

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

In accordance with Invitek Molecular's EN ISO 13485 certified Quality Management System the performance of all components of the **InviMag® Stool DNA Kit/ KF96** have been tested separately against predetermined specifications routinely on lot-to-lot to ensure consistent product quality.

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

For technical support or further information, please contact:

from Germany: +49-(0)30-9489-2901/ 2910
from abroad: +49-(0)30-9489-2907 or contact your local distributor.

Intended use

The **InviMag® Stool DNA Kit/ KF96** is designed for fast and efficient purification of microbial and genomic DNA from fresh and frozen human or animal stool samples or from sample types with high concentrations of PCR inhibiting components.

An important application for the kit is the use in metagenomics research for specification of gut bacteria.

Stool samples typically contain many sources that can degrade DNA and inhibit downstream enzymatic reactions. The **InviMag® Stool DNA Kit/ KF96** contains optimized wash buffers for efficient inhibitor removal.

The **InviMag®** technology combines the advantages of the innovative **Invisorb®** technology for isolation of genomic DNA (without chaotropic buffer components) with easy handling of magnetic particles for an efficient and reliable purification of genomic DNA. The **InviMag® Stool DNA Kit/ KF96** is designed for an optimal use on a KF96 or KFflex96 instrument from Thermo Scientific.

THE PRODUCT IS INDENTED FOR USE BY PROFESSIONALS ONLY, SUCH AS TECHNICIANS, PHYSICIANS AND BIOLOGISTS TRAINED IN MOLECULAR BIOLOGICAL TECHNIQUES. It is designed to be used with any downstream application employing enzymatic amplification or 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 about other clinical or laboratory findings.

To minimize irregularities in 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, nor for the purification of RNA or viral nucleic acids.

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, implied or expressed.

The user is responsible to validate the performance of the Invitek Molecular product for any particular use. Invitek Molecular does not provide for validation of performance characteristics of the product with respect to specific applications.

Invitek Molecular products may be used e.g. in clinical diagnostic laboratory systems under following conditions:

- If used in the US, based on the condition that the complete diagnostic system of the laboratory has been validated pursuant to CLIA' 88 regulations.
- For other countries based on the condition that the laboratory has been validated pursuant to equivalents according to the respective legal basis.

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

The chemicals and the plastics are for laboratory use only. They have to be stored in the laboratory and must not be used for other purposes than intended.

The product and its contents are not suitable for consumption.

Safety information

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

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

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-molecular.com for each Invitek 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.

Invitek Molecular has not tested the liquid waste generated by the **InviMag® Stool DNA Kit/ KF96** procedures for residual infectious materials. Contaminations 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 accordingly to local safety regulations.

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

Lysis Buffer P

Warning

H319 –H412.-P280- P305-351-338-P273

Wash Buffer I

Warning

H302-H412-P280-P305-P351-P338-P273-EUH032

Proteinase K

Danger

H315-319-334-335 P280-P305-P351-P338

H302: Harmful if swallowed.
H315: Causes skin irritation.H319: Causes serious eye irritation.
H319: Causes serious eye irritation.
H334: May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335: May cause respiratory irritation.
P273: Avoid release to the environment.
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.
EUH032: Contact with acids liberates very toxic gas.

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

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

Product characteristic of the InviMag® Stool DNA Kit/ KF96

Starting material	Yield	Time	Ratio
200-400 mg fecal sample	up to 50 µg (depends on starting material)	about 50 min (incl. lysis time)	OD_{260}/OD_{280} 1.6–1.8

The **InviMag® Stool DNA Kit/ KF96** allows a semi-automated rapid and efficient isolation of high quality total DNA from up to 200–400 mg of fresh or frozen human and animal stool samples by combining of an efficient lysis of starting material, a removal of PCR inhibitors with the binding of DNA onto magnetic particles. The process is a standardized procedure, reducing mistakes in analysis.

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

Stool samples typically contain many inhibitors that can degrade DNA and inhibit downstream enzymatic reactions. During the transportation of stool samples at room temperature, the DNA will be massively degraded, and the pathogen load is changing during transportation. To prevent this, Invitek Molecular offers special transport containers with Stool DNA Stabilizer. The Stool Collection Tubes contain 8 ml of Stool DNA Stabilizer, a buffer formulation, which prevents DNA degradation, stabilizes the present microorganisms, enables prelysis of the sample and will stabilize the DNA for at least 3 months at ambient temperature. Beside saving and stabilization of traces of human DNA, the Stool DNA Stabilizer is also very successful if bacterial pathogens should be detected, which are difficult to lyse due to their cell wall structure. These Stool Collection Tubes with Stool DNA Stabilizer can be ordered separately (see page 266).

A rigorous prelysis step using **Zirconia beads** with optimized prelysis buffer in a temperature step is followed by preincubation of the sample with **InviAdsorb** to remove PCR inhibitors. Undissolved particles and PCR inhibitors will bind to **InviAdsorb** and are removed by a subsequent centrifugation step. The following **Proteinase K** digestion ensures high yields especially for Gram-positive gut bacteria. Stool contains a large range of DNA like host DNA from colon epithelial cells, parasite DNA, bacterial DNA, DNA from food or DNA from gastrointestinal pathogens. The feasibility to select between different lysis conditions allows the enrichment or reduction of the content of bacterial DNA compared to the host. The **InviMag® Stool DNA Kit/ KF96** uses optimized washing conditions for inhibitor removal. At the end, 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 like:

- PCR applications
- Hybridization
- Genetic typing
- Pathogen typing
- Mutation analysis
- Paternity analysis

To increase the robustness of PCR assays using DNA isolated from stool samples, the addition of BSA to a final concentration of 0.1 µg/µl to the PCR mixtures is recommended. In eluates derived from feces, the ratio of target DNA to background DNA is often very low.

Principle and procedure

The **InviMag® Stool DNA Kit/ KF96** procedure comprises following steps:

- Stabilization and lysis 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 P or Stool DNA Stabilizer, which inactivates DNases, the human cells and the bacterial cell walls will be lysed (depending on the temperature profile, in combination with beads for enrichment of bacterial DNA). The lysate will be mixed with **InviAdsorb** and most of the PCR inhibiting components will be removed. After lysis, the DNA binds to the magnetic beads whereas contaminations and enzyme inhibitors are efficiently removed by washing steps. At the end of the process, highly purified DNA is eluted in **Elution Buffer**.

This manual contains 3 protocols.

Sampling and storage of starting material

The collected fresh stool sample can be stored at ambient temperature for at least 1-2 hours at room temperature, but the high content of DNases will result in a quick DNA digestion and degradation. The sample should therefore be quickly added to the lysis buffer or can be stored frozen -20°C for several weeks.

The storage of fresh samples in Stool DNA Stabilizer allows storage at room temperature for about 3 months. The storage of fresh samples in Stool DNA Stabilizer will lead to less degraded DNA and a better yield of difficult to lyse bacterial pathogens. The storage time has no influence on the amount of host cell DNA.

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

The collected sample should 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).

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

Procedure

Lysis

Stool samples are lysed in **Lysis Buffer P** under denaturing conditions at elevated temperatures. Human cells lyse efficiently at room temperature whereas bacterial cells and those of other pathogens in the stool sample are efficiently lysed by incubation at 95°C. This procedure is recommended for 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°C and the ratio of non-human to human DNA will increase significantly.*

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 a prefilled safe-look tube. The matrix with the bound contaminations and cell debris are pelleted by centrifugation while the DNA in the supernatant is pre-cleaned and separated.

Protein digestion

Proteinase K is added to the supernatant to digest and degrade proteins during incubation at 70°C.

Binding of total DNA

After adding **Binding Buffer A** to the supernatant, the mixture is mixed with **MAP Solution A** (magnetic beads) and the nucleic acids are bound to the beads.

Optimal salt concentrations and pH conditions in the lysate ensure that remains of digested proteins and other contaminations, which can inhibit downstream enzymatic reactions, are not retained on the beads.

Removing residual contaminants

Contaminants are efficiently and completely removed using **Wash Buffer I** and **II**, while the nucleic acids remain bound to the beads.

Elution

The nucleic acids are eluted in low salt using **Elution Buffer** and are ready-to-use in different subsequent tests.

Yield and quality of genomic DNA

The amount of purified DNA in the **InviMag® Stool DNA Kit/ KF96** procedure from feces depends on the healthy status of the donor, bacteria content, sample source, transport, storage and age. A typical yield is about 10 - 80 µg. Yield and quality of isolated genomic DNA is suitable for any molecular-diagnostic detection system. The diagnostic tests should be performed accordingly to the manufacturers' specifications.

Important notes

Important points before starting a protocol

Immediately upon receipt of the product, inspect the all components for any apparent damage, correct quantity and quality. If there are any unconformities, please notify Invitek Molecular in writing with immediate effect upon inspection thereof. If buffer bottles are damaged, contact the Invitek Molecular Technical Services or your local distributor. In case of liquid spillage, refer to "Safety Information" (see page 6). Do not use damaged kit components because their use may lead to poor kit performance.

- Always change pipet tips between different liquid transfers. To avoid cross-contaminations 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 contaminated gloves immediately.
- 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 pathogen 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

5x 96 total DNA-extractions:
Add 56 ml 99.7% Isopropanol to each **Binding Buffer A.** Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times.
Add 1.5 ml ddH_2O to each **Proteinase K**, mix thoroughly until completely dissolving!
Add 80 ml 96-100% ethanol to each bottle **Wash Buffer I.**
Add 140 ml 96-100% ethanol to each 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 webpage: www.invitek-molecular.com)

- o Microcentrifuge
- o Thermomixer (for 95°C)
- o Measuring cylinder (250 ml)
- o Disposable gloves
- o Pipet with tips
- o Reagents reservoirs for multichannel pipets
- o 96-100% ethanol
- o ddH_2O
- o Vortexer
- o Isopropanol*

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

*** Possible suppliers for Isopropanol:**

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

Applichem
2-Propanol für die Molekularbiologie
Order no. A3928

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

Important indications

1. The kit procedure is suitable for purifying DNA from very small amounts of starting material. If the sample contains less than 5 ng DNA (>1.000 copies), 3-5 µg Carrier (a homopolymer such as poly–dA, poly-dT or gDNA) should be added to the starting material. Ensure that the Carrier-DNA does not interfere with other downstream applications. Alternatively, the Carrier-RNA can be degraded by RNase digestion after purification. The carrier should be added to the lysis buffer before preparation or to the stabilization buffer – stool mixture. Never add the carrier directly to the stool sample.
2. **MAP Solution A** will always co-purify low amounts of RNA beside DNA. For the elimination of RNA (if necessary) add 20 µl RNase A (10 mg/ml) before adding **Binding Buffer A**. Incubate the sample at room temperature for 5 min. Continue as described in the corresponding protocol.

Elution of DNA

- ○ For downstream applications that require small staring volumes, a more concentrated eluate may increase assay sensitivity. The elution can be done by using a lower volume of **Elution Buffer** (down to 75 µl). This may result in a higher concentration of DNA. Lower volumes of **Elution Buffer** will decrease the overall yield of DNA!
- ○ If low concentrated Tris buffer affects sensitive downstream applications, use water for the elution step. However, ensure that the pH of the water is at least 7.0 (deionized water from certain sources can be slightly acidic). DNA stored in water may be subjected to degradation by acid hydrolysis.
- ○ Eluting twice with each 75 µl **Elution Buffer** is also possible and produces slightly higher yields of DNA. However, a changed elution volume or performing the elution twice with a low volume demands a modification of the assay file within the BindIt software!

Scheme of the InviMag® Stool DNA Kit/ KF96

Please read the protocols carefully before starting any preparation

Transfer 200 mg of the stool sample into a 2 ml reaction tube (not provided)
Add 1.2 ml **Lysis Buffer P** to the sample and vortex for 1 min.

For enrichment of host DNA: Incubate for 10 min at RT while shaking
For enrichment of bacterial DNA: Incubate for 10 min at 95°C on a thermomixer while shaking
Add 5 **Zirconia Beads II** to the homogenate and vortex for 2 min

During lysis, prefill all plates with required buffers and appropriate volumes

Tip Plate: Place the KF 96 Tip Comb for DW magnets on a 200 µl **Elution Plate (**Elution Plates and Tip Plates are identically. Use one provided Elution Plate as a Tip Plate)

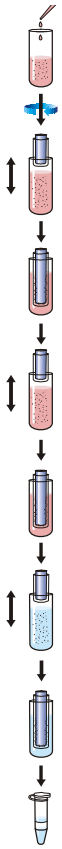
Binding Plate: Add 25 µl **Proteinase K** to a 2 ml Deep Well Plate
Washing Plate 1: 800 µl **Wash Buffer I** to a 2 ml Deep Well Plate
Washing Plate 2: 800 µl **Wash Buffer II** to a 2 ml Deep Well Plate
Washing Plate 3: 800 µl **Wash Buffer II** to a 2 ml Deep Well Plate
Elution Plate: 150 µl **Elution Buffer** to a KF Elution Plate

Centrifuge the lysed sample at 13.400 x *g* (12.000 rpm) for 1 min

Transfer the supernatant into an **InviAdsorb Tube** and mix by vortexing for 10 s.
Incubate for 1 min at RT and centrifuge for 3 min at max. speed (13000 rpm)

Transfer 400 µl from the supernatant into the Binding Plate, prefilled with 25 µl **Proteinase K**.
Start the run (for detailed information see chapter “Starting a run”, page 17).

After lysis, a pause occurs and **300 µl Binding Buffer A** *(follow preparing instructions)* and **20 µl MAP Solution A** have to be added to the Binding Plate



Proteinase K digestion

DNA binding to magnetic particle

Magnet bead separation

3x Washing of the particle fixed DNA

3x Magnet bead separation

Ethanol Removal

Elution of genomic DNA

Removal of beads

Pure total DNA

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.

Attention: *Please be aware, that the* ***Binding Buffer A*** *has to be prepared– see instruction page: 10*

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

1. Sample Lysis

Weigh in 200-400 mg of **stool sample** (fresh or frozen) into a 2.0 ml reaction tube (not provided). Add 1.2 ml **Lysis Buffer P** to each stool sample and vortex vigorously for 1 min.

Important: *If the sample is liquid, transfer 200 µl into a 2.0 ml reaction tube. Cut-off the end of the pipet tip to relieve pipetting. If the sample is frozen, use a scalpel or spatula to scrape bits of stool into a 2.0 ml reaction tube on ice. Ensure that the samples do not thaw until Lysis Buffer P is added; otherwise, the sample containing nucleic acids may degrade.*

For an enrichment of bacterial DNA:

Incubate the sample for 10 min at 95°C in a thermomixer while continuously shaking at 900 rpm.

Add 5 Zirconia Beads II to the homogenate and vortex for 2 min at room temperature.

Centrifuge the sample at 13.200 x *g* (12.000 rpm) for 1 min to pellet solid stool particles.

For an enrichment of host DNA

Incubate the sample for 10 min at RT while continuously shaking at 900 rpm.

Centrifuge the sample at 13.200 x *g* (12.000 rpm) for 1 min to pellet solid stool particles.

Note: ***During sample lysis, prefill all plates with the required buffers and appropriate volumes.***

2. Removal of PCR Inhibitors

Transfer the supernatant into an InviAdsorb-Tube and vortex vigorously for 15 s.

Incubate the suspension for 1 min at room temperature. Centrifuge the sample at full speed (13000 rpm) for 3 min.

3. Protein Digestion

Transfer 25 µl **Proteinase K** into the Binding Plate and add 400 µl from the sample supernatant from step 2.

Continue with the category “Starting a run on a KingFisher instrument” on page 17.

Protocol 2: Isolation of total DNA from 200 mg stool samples with extraction of difficult to lyse bacteria

Please read the instructions carefully and conduct the prepared procedure.

Attention: *Please be aware, that the* ***Binding Buffer A*** *has to be prepared– see instruction page: 10*

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

***Important Note*:** *To completely lyse some special bacteria, (like Mycobacterium paratuberculosis or Chlamydia) a specific treatment is required*

Set the heating blocks (e.g. thermomixer) to 70°C and 95 °C

Prepare a container with crushed ice

Important Note: *Addition of zirconia beads (e.g. 5-10 beads with a diameter of 1 mm) to each stool sample /lysis buffer mixture or stool in stabilizer mixture may improve lysis.*

Vortex the sample during all incubation steps for 5-7 times.

1. Sample homogenization and prelysis

- Weigh in 200 mg or 200 µl of the stool sample (fresh or frozen) into a 2.0 ml reaction tube (not provided) and add 1.2 ml Lysis Buffer P. Vortex vigorously for 1 min.
- Incubate the homogenized sample for 10 min at 95°C in a thermomixer while continuously shaking at 900 rpm.
- Incubate the sample on ice for 3 min.
- Add 5–10 Zirconia Beads II to the homogenate.
- Incubate the sample at 95°C for 3 min, followed by a vortexing step for 1 min.
- Centrifuge the sample at 13.200 x *g* (12.000 rpm) for 1 min to pellet solid stool particles.

2. Removal of PCR Inhibitors

Transfer the supernatant into an InviAdsorb-Tube and vortex vigorously for 15 s.

Incubate the suspension for 1 min at room temperature. Centrifuge the sample at full speed (13.000 rpm) for 3 min.

3. Protein Digestion

Transfer 25 µl **Proteinase K** into the Binding Plate and add 400 µl of the sample supernatant from step 2.

Continue with the category “Starting a run on a KingFisher instrument” on page 17.

Protocol 3: 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.

Attention: *Please be aware, that the* ***Binding Buffer A*** *has to be prepared– see instruction page: 10*

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

Stool Collection Tubes are not provided in the kit (see ordering info, page 26)

1. Collection of the stool sample and stabilization

Note: *The Stool Collection Tubes (not provided, must be ordered separately) contain 8 ml of Stool Stabilizer. That is a new developed buffer formulation, which enables the prelysis of the sample and stabilization of the DNA for at least 3 months 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) from the fresh stool sample.
2. Transfer the spoon containing 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.

Important Notes:

The collected sample can be stored at ambient temperature for at least 3 months. The storage in ***Stool DNA Stabilizer*** *will lead to 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 can also be used immediately after collection for the isolation of DNA.

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. Sample homogenization and prelysis

Transfer 1.4 ml of the collected and well-homogenized stool sample (Stool DNA Stabilizer with stool specimen) after storage or directly after collection into a 2.0 ml reaction tube.

For an enrichment of host DNA, do not perform this temperature step.

Centrifuge the sample at 13.200 x *g* (12.000 rpm) for 1 min to pellet solid stool particles.
This will lead to a reduced amount of extracted total DNA but will not influence the amount of human DNA

For an enrichment of bacterial DNA:

Incubate the homogenized sample for 10 min at 95°C in a thermomixer while continuously shaking at 900 rpm.
Add 5 Zirconia Beads II to the homogenate and vortex for 2 min at RT.
Centrifuge the sample at 13.200 x *g* (12.000 rpm) for 1 min to pellet solid stool particles.

Important Note: *The incubation step at 95°C will maximize the yield of bacterial DNA because of a very efficient destruction of the cell wall of e.g. gram$^+$ bacteria.*

3. Removal of PCR Inhibitors

Transfer the supernatant into an InviAdsorb tube and vortex vigorously for 15 s.

Incubate the suspension for 1 min at room temperature. Afterwards centrifuge the sample at full speed (13000 rpm) for 3 min.

4. Protein Digestion

Transfer 25 µl **Proteinase K** into the Binding Plate and add 400 µl of the sample supernatant from step 2. Continue with the category “Starting a run on a KingFisher instrument” on page 17.

Additional Protocol 4: Post purification of DNA containing inhibitors

***Important Note*:** *Stool samples are very heterologous, depending on the host and source of stool. In some cases, inhibitors for downstream reactions might occur in the eluted DNA. In this case, following the post purifying protocol may help.*

1. Eluate adjustment

Adjust your eluate to at least 100 µl using distilled water.

2. Sephadex G50 Slurry

Make slurry of Sephadex G50 by adding water to the Sephadex G50 powder until the slurry is reaching its final extension (max. 30 min).

3. Adsorption of inhibitors

Add the amount of 1/3 from the prepared slurry to the eluate. Incubate for 30 min while continuously shaking at room temperature (RT).

4. Removal of slurry

Centrifuge the mixture at 10.000 x *g* (10.500 rpm) and transfer the supernatant (containing the DNA) into a new reaction tube.

This purification may be repeated once, but remember that you will lose about 25% of your yield for every purification step.

Starting a run on a KF96 / KFflex96 instrument

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

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

Tip Plate:	Place **KF96 Tip Comb** for DW magnets on 200 µl Elution Plate
Binding Plate:	Add 25 µl **Proteinase K** to a 2 ml Deep Well Plate
Washing Plate 1:	Add 800 µl **Wash Buffer I** to a 2 ml Deep Well Plate
Washing Plate 2:	Add 800 µl **Wash Buffer II** to a 2 ml Deep Well Plate
Washing Plate 3:	Add 800 µl **Wash Buffer II** to a 2 ml Deep Well Plate
Elution Plate:	Add 150 µl **Elution Buffer** to the KF Elution Plate

- Transfer 400 µl from the **cleared sample** into the Binding Plate containing 25 µl **Proteinase K**.
- Start the purification program by either selecting the assay "**InviMag Stool DNA KF96**" or "**InviMag Stool DNA KFflex96**" depending on the instrument you are using.
- Insert the prefilled plates onto the right position of the instrument surface by following the specification printed on display and confirm every step by pressing the "START" button.
- After a 20 min lysis step, a pause occurs and 300 µl **Binding Buffer A** and 20 µl **MAP Solution A** have to be added to sample containing cavities of the Binding Plate. After addition, reinsert the plate into instrument (watch the correct plate orientation) and continue the run by pressing the "START" button. The instrument will continue with the extraction / purification process without any further user interference.
- After the run, the elution plate contains the isolated nucleic acids. The eluates can now be transferred into the provided 1.5 ml receiver tubes or be used directly from the elution plate.

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

For self-programming of the KF96 and KFflex96 instrument

Reagent info

Tip Plate		KingFisher 96 KF plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
-	-	-	-

Binding Plate		Microtiter DW 96 plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Prelysed Sample	400	-	Sample
Proteinase K	25	-	Reagent

Washing Plate 1		Microtiter DW 96 plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Wash Buffer I	800	-	Reagent

Washing Plate 2		Microtiter DW 96 plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Wash Buffer II	800	-	Reagent

Washing Plate 3		Microtiter DW 96 plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Wash Buffer II	800	-	Reagent

Elution Plate		KingFisher 96 KF plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Elution Buffer	150	-	Reagent

Dispensed reagents

Binding Plate		Microtiter DW 96 plate	
Name	**Step**	**Well volume [µl]**	**Total reagent volume [µl]**
Binding Buffer A	Adjust Binding	300	-
MAP Solution A	Adjust Binding	20	-

Steps data

	Tip	96 DW tip comb	
	Pick-Up	Tip Plate	
	Heating	Binding Plate	
	Beginning of step	Precollect	No
		Release beads	Yes
	Mixing / heating:	Mixing time, speed	00:20:00, Medium
		Heating temperature [°C]	60
		Preheat	Yes
	End of step	Postmix	No
		Collect beads	No
	Adjust Binding	Binding Plate	
		Message	Add Binding Buffer A and MAP Solution A
		Dispensing volume [µl]	320
	Reagent(s)	Name	Binding Buffer A
		Volume [µl]	300
		Name	MAP Solution A
		Volume [µl]	20
	Binding	Binding Plate	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:05:00, Medium
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	4
		Collect time [s]	5
	Washing_1	Washing Plate 1	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:01:30, Fast
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	4
		Collect time [s]	5
	Washing_2	Washing Plate 2	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:01:00, Fast
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	4
		Collect time [s]	5

	Washing_3	Washing Plate 3	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:01:00, Fast
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	4
		Collect time [s]	5
	Drying	Washing Plate 3	
		Dry time	00:05:00
		Tip position	Outside well / tube
	Elution	Elution Plate	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Medium
	Mixing / heating:	Mixing time, speed	00:07:30, Slow
		Heating temperature [°C]	60
		Preheat	No
	End of step	Postmix	No
		Collect count	5
		Collect time [s]	5
	Bead Removal	Washing Plate 3	
		Release time, speed	00:00:30, Fast
	Leave	Tip Plate	

Troubleshooting

Problem	Comments and suggestions
low amount or no DNA of extracted DNA	
sample stored incorrectly	sample should be stored at 4°C or -20°C
insufficient homogenization of stool sample in **Lysis Buffer P** or in **Stool DNA Stabilizer**	repeat the DNA purification procedure with a new sample Be sure to mix the sample and **Lysis Buffer P** or in **Stool DNA Stabilizer** until the sample is thoroughly homogenized use Zirconia beads and vortex for homogenization
insufficient lysis	increase lysis time reduce amount of starting material overloading of Spin Filter reduces yield!
insufficient mixing of the sample with **Binding Buffer A**	mix sample sufficient by pipetting up and down with **Binding Buffer A** prior to transfer the sample to the beads
no alcohol added to the **Wash Buffer I** and **II**	check that **Wash Buffer I** and **Wash Buffer II** concentrates were diluted with correct volume of 96-100% ethanol. repeat the purification procedure with a new sample
DNA not eluted efficiently	incubate the beads for 5 min at room temperature before elution
low amount of **MAP Solution A**	mix **MAP Solution A** thoroughly before pipetting to the KingFisher tube
A260/A280 ratio for purified nucleic acids is low	
inefficient elimination of inhibitory substances due to insufficient mixing with the **InviAdsorb** matrix	repeat the DNA purification procedure with a new sample be sure to mix the sample and **InviAdsorb** matrix until the sample is thoroughly homogenized
insufficient mixing with **Lysis Buffer P**	repeat the procedure with a new sample be sure to mix the sample and **Lysis Buffer P** immediately and thoroughly by pulse vortexing
decreased **proteinase** activity	repeat the DNA purification procedure with a new sample and with **Proteinase K** for difficult cases use double volume **Proteinase K**
no **Binding Buffer A** added to the lysate	repeat the purification procedure with a sample
Wash Buffer I and **Wash Buffer II** prepared incorrectly	check that **Wash Buffer I** and **Wash Buffer II** were diluted with 96–100% ethanol do not use denatured alcohol, which contains other substances such as methanol or methylethylketone repeat the purification procedure with a new sample
Wash Buffer I and **Wash Buffer II** used in the wrong order	ensure that **Wash Buffer I** and **Wash Buffer II** are used in the correct order in the protocol
protein contamination	repeat washing step with **Wash Buffer I** in the repeated preparation

DNA does not perform well in downstream applications	
BSA not added to PCR mixture	When using eluates in PCR, for maximum PCR robustness add BSA or I-Solution to a final concentration of 0.1 µg /µl to the PCR mixture.
too much DNA used in downstream reaction	The **InviMag® Stool DNA Kit /KF96** purifies total DNA, which could originate from many different organisms present in the original stool sample (e.g., human, animal, plant, bacterial). If the amount of total DNA is too high, PCR could be inhibited by excess total DNA. Reduce the amount of eluate used in the downstream reaction if possible
nonspecific bands in	It is likely that only a low quantity of target downstream PCR DNA is present in stool-sample eluates, together with high amounts of background DNA.
inefficient lysis of target cells	The amount of target DNA in the eluate may be low if the target cells are difficult to lyse, as is the case with some bacteria and parasites. In future preparations, prolong incubation time of the sample at 95°C and/or add zirconia beads to the stool samples lysis mixture (see **InviMag® Stool DNA Mini Kit/ KF96**, protocol 1.)
not enough DNA in eluate	check "**Low amount or no DNA of extracted DNA**" for possible reasons.
inhibitory substances in preparation	See "A_{260}/A_{280} ratio for purified nucleic acids is low" for possible reasons. Bring the eluate volume to 200 µl .add to the supernatant 400 µl **Lysis Buffer P** and mix all with 200 µl **Binding Buffer A**. Repeat the protocol 1 from step 5 of "Isolation of total DNA from up to 200 mg stool samples with and without enrichment of bacterial DNA " (page 133). **See also protocol: Post Purification**
residual **Wash Buffer** in the eluate	Ensure that the **Wash Buffer I** and **II** are used in the correct order in the protocol. add 400 µl **Lysis Buffer P** and 200 µl **Binding Buffer A** to the eluate, and continue with step 5 of "Protocol: Isolation of total DNA from up to 200 mg stool samples with and without enrichment of bacterial DNA " (page 13).
residual **MAP Solution A** in the eluate	transfer the eluate in a 1.5 ml Tube, spin down at maximum speed for 2 min and transfer the supernatant in a new tube
insufficient mixing with Lysis Buffer P or Stool DNA Stabilizer	repeat the purification with other aliquots
reduced sensitivity of amplification reaction	Determine the maximum volume of eluate amplification reaction suitable for your amplification reaction. Reduce or increase the volume of eluate added to the reaction optimize your amplification system e.g. by changing template volume
Little or no supernatant visible after initial centrifugation step	Increase the centrifugation time proportionately if your centrifuge cannot provide 13.400 x *g* (12.000 rpm), e.g. instead of centrifuging for 1 min at 13.400 x *g*, centrifuge for 3 min at 10.000 x *g*).
insufficient centrifugal force	

Little or no supernatant visible after centrifugation step with InviAdsorb matrix insufficient centrifugation force	With some samples, centrifugation to precipitate the **InviAdsorb** matrix may result in a pellet that is not sufficiently compact. in these cases, it is recommended to increase the centrifugation time for precipitation of **InviAdsorb** matrix to 6 minutes.
Precipitate after addition of Binding Buffer A	In most cases, this effect forms big amounts of DNA in the sample. Don't remove this precipitate and follow strictly the protocol.

Appendix

KingFisher™ BindIt Software 3.2 or higher versions

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

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

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

Minimum system requirements for BindIt Software 3.2 or higher versions

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

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

General notes on handling DNA

Nature of DNA

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

Storage of DNA

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

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

Drying, dissolving and pipetting DNA

Avoid over-drying of genomic DNA after ethanol precipitation. It is better to 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 on sample source, transport, storage and age.

Ordering information

Product	Package size	Catalogue No.
InviMag® Stool DNA Kit/ KF96	5 x 96 purifications	7438300200

Related products

PSP® Spin Stool DNA Kit	250 extractions	1038100300
PSP® Spin Stool DNA *Plus* Kit	250 purifications	1038110300
PSP® Spin Stool DNA Basic Kit	250 purifications	1038120300
Stool Collection Tubes	250 tubes	1038111300

KingFisher™ 96 and consumables

KingFisher 96, Magnetic Particle Processor,100-240V,50/60Hz (including one magnetic head)	5400500
KingFisher 96 Head for Deep Well plate	24073430
KingFisher 96 tip comb for PCR magnets, 8 x 10 pcs / box	97002514
KingFisher 96 tip comb for KF magnets, 10 x 10 pcs / box	97002524
KingFisher 96 tip comb for DW magnets 10 x 10 pcs / box	97002534
KingFisher 96 KF plate (200ul) 48 plates / box	97002540
Microtiter deep well 96 plate, 50 plates/box	95040450

Possible suppliers for Isopropanol:

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

Applichem
2-Propanol für die Molekularbiologie
Order no. A3928

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

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