Instruction InviMag® Stool DNA Kit /KF96 /KFflex 96

The **InviMag® Stool DNA Kit /KF96 /KFflex 96** is the ideal tool using the Invisorb® technology in combination with magnetic beads for rapid and efficient isolation and purification of high purity DNA from max. 200 mg of fresh or frozen stool sample. The purified DNA is of high quality and well suited for use in *in-vitro* diagnostic analysis.

The kit is very useful for metagenomic analysis of stool samples as well for all other relevant diagnostic applications.

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 or the purification of 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.

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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.

InviMag® is a registered trademark of STRATEC Molecular GmbH.
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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Contents

Kit contents of the InviMag® Stool DNA Kit /KF96 /KFflex 96 3

Kit contents of the InviMag® Stool DNA Kit /KF96 /KFflex 96 / wp 4

Symbols 5

Storage 5

Quality control 5

Intended use 6

Product use limitation 6

Safety information 7

Product characteristic of the InviMag® Stool DNA Kit /KF96 /KFflex 96 8

Principle and procedure 9

Sampling and storage of starting material 9

Procedure 10

Yield and quality of the genomic DNA 10

Important notes 11

Preparing reagents and buffers 11

Equipment and reagents to be supplied by user 12

Scheme of the InviMag® Stool DNA Kit /KF96 /KFflex 96 13

Protocol 1: Isolation of genomic DNA from up to 200 mg stool samples with and without enrichment of bacterial DNA 14

Protocol 2: Isolation of total DNA from 200 mg stool samples from difficult to lyse bacteria 15

Protocol 3: Isolation of genomic DNA from stabilized stool samples with and without enrichment of bacterial DNA 16

Additional Protocol 4: Post purification of DNA containing inhibitors 17

For self programming of the KingFisher 96 System 19

Troubleshooting 24

Appendicies 27

General notes on handling DNA 28

Order information 29

Kit contents of the InviMag® Stool DNA Kit /KF96 /KFflex 96

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

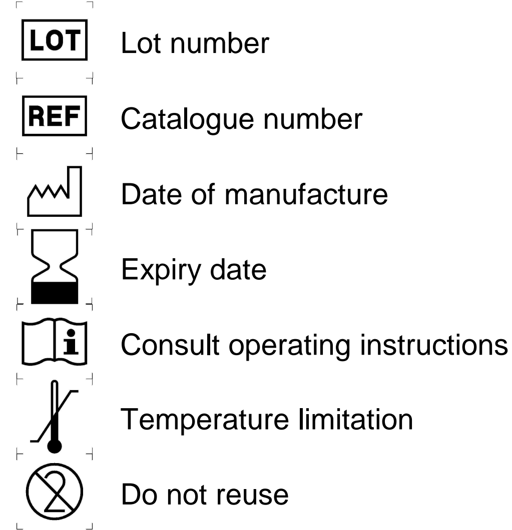
	1 x 96 extractions	5 x 96 extractions
Catalogue Number	7438300100	7438300200
Lysis Buffer P	125 ml	3 x 210 ml
Proteinase K	for 2 x 1.5 ml working solution	for 13 ml working solution
InviAdsorb	96	5 x 96
Zirconia Beads II	3 vials	14 vials
MAP Solution A	2 x 1.1 ml	10.5 ml
Binding Buffer P	30 ml	160 ml
Wash Buffer I	80 ml (final volume 160 ml)	3 x 80 ml (final volume 3x 160 ml)
Wash Buffer II	60 ml (final volume 200 ml)	5 x 60 ml (final volume 5x 200 ml)
Elution Buffer D	30 ml	120 ml
1.5 ml Receiver Tube	2 x 50	10 x 50
2,0 ml Deep Well Plate	4	20
KF 96 Tip Comb for DW magnets	1	5
200 µl Elution Plate	2	10
Manual	1	1
Initial steps	Add 80 ml of 96-100% ethanol to the bottle **Wash Buffer I**, mix thoroughly and keep the bottle always firmly closed! Add 140 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 1.5 ml of ddH_2O, to each vial, mix thoroughly and store like described!	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 **Proteinase K** by addition of 13 ml of ddH_2O, to the respective bottle mix thoroughly and store like described!

Kit contents of the InviMag® Stool DNA Kit /KF96 /KFflex 96 / wp

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

	1 x 96 extractions	**5 x 96 extractions**
Catalogue Number	7438300150	7438300250
Lysis Buffer P	125 ml	3 x 210 ml
Proteinase K	for 2 x 1.5 ml working solution	for 13 ml working solution
InviAdsorb	96	5 x 96
Zirconia Beads II	3 vials	14 vials
MAP Solution A	2 x 1.1 ml	10.5 ml
Binding Buffer P	30 ml	160 ml
Wash Buffer I	80 ml (final volume 160 ml)	3 x 80 ml (final volume 3x 160 ml)
Wash Buffer II	60 ml (final volume 200 ml)	5 x 60 ml (final volume 5x 200 ml)
Elution Buffer D	30 ml	120 ml
1.5 ml Receiver Tube	2 x 50	10 x 50
Manual	1	1
Initial steps	Add 80 ml of 96-100% ethanol to the bottle **Wash Buffer I**, mix thoroughly and keep the bottle always firmly closed! Add 140 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 1.5 ml of ddH_2O, to each vial, mix thoroughly and store like described!	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 **Proteinase K** by addition of 13 ml of ddH_2O, to the respective bottle mix thoroughly and store like described!
Plastic to be supplied by user (see order information)		
2,0 ml Deep Well Plate	4	20
KF 96 Tip Comb for DW magnets	1	5
200 µl Elution Plate	2	10

Symbols



Storage

All buffers and kit contents of the **InviMag® Stool DNA Kit /KF96 /KFflex 96**, except **Proteinase K** and **MAP Solution A** should be stored at room temperature and are stable for at least 12 months under these conditions.

Proteinase K

Lyophilized Proteinase K should be stored at 2-8°C.

Dissolved Proteinase K must be stored at -20°C. Rep eated freezing and thawing will reduce the activity of Proteinase K dramatically. Dividing Proteinase K into aliquots and storage at -20°C is recommended.

MAP Solution A

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.

Room temperature is defined as range from 15–30°C.

Quality control

STRATEC Molecular guarantees the correct function of the **InviMag® Stool DNA Kit /KF96 /KFflex 96**, for applications as described in the manual. In accordance with STRATEC Molecular's certified QM-System each component of the **InviMag® Stool DNA Kit /KF96 /KFflex 96**, 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 /KF96 /KFflex 96**, or other STRATEC Molecular products, please do not hesitate to contact us.

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/ KFflex 96** has been designed for fast and efficient purification of microbial and genomic DNA from fresh and frozen human or animal stool samples or from other 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 compounds that can degrade DNA and inhibit downstream enzymatic reactions. The **InviMag® Stool DNA Kit /KF96 /KFflex 96,** optimized the essential washing conditions to remove all potent inhibitors very efficiently.

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 genomic DNA. The **InviMag® Stool DNA Kit /KF96 /KFflex 96** 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, nor for the purification of RNA. The application of the kit for isolation and purification of viral DNA has not been evaluated.

The included chemicals are only useable once.

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 (MSDSs). 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 /KF96 /KFflex 96** 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 regulation.

Below is listed European Community risk and safety phrases for the components of the **InviMag® Stool DNA Kit /KF96 /KFflex 96** to which they apply.

Lysis Buffer P

danger

H319 P305-351-338

Proteinase K:

danger

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

Binding Buffer P

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

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 /KF96 /KFflex 96

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} 1.4–1.8

The **InviMag® Stool DNA Kit /KF96 /KFflex 96** 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 sample by combining of an efficient lysis of starting material, an efficient removal of PCR inhibitors with the very efficient binding of DNA onto magnetic particles. 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 contain 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: 10381112).

A rigorous prelysis steps using **Zirconia beads** with optimized prelysis buffer under high temperatures, is followed by preincubation of the sample with **InviAdsorb** to remove PCR inhibitors. Undissolved particles and PCR inhibitors bound to **InviAdsorb** are removed by a centrifugation step. The following **Proteinase K** digestion ensures high yields especially also from gram positive gut bacteria. 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 /KF96 /KFflex 96** 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

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/ KF96** cannot be construed as an authorization or implicit licence to practice PCR under any patents held by Hoffmann-LaRoche Inc

No toxic or hazardous chemicals like phenol/chloroform or ß-Mercaptoethanol are used. Traditional time-killing procedures can be replaced using the InviMag® Stool DNA Kit /KF96 /KFflex 96**.**

To increase 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 DNA eluates from feces, the ratio of target DNA to background DNA is often very low. To increase the specificity of downstream PCR assays, the use of the **InviTaq Hot Start DNA Polymerase** (see ordering information's, page 27) is recommended. The combination of the **InviMag® Stool DNA Kit /KF96 /KFflex 96** sample preparation and the use of BSA and InviTaq Hot Start DNA Polymerase in downstream amplification maximizes PCR robustness and specificity when amplifying DNA prepared from stool samples.

Principle and procedure

The **InviMag® Stool DNA Kit /KF96 /KFflex 96** 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 inactivate DNases, the human cells and the bacterial cell wall will be lysed more or less (depending from the temperature profile, in combination with beads for enrichment of bacterial DNA). The lysate will be mixed with **InviAdsorb** and the most PCR inhibiting components will be removed, 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 D** or water.

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 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℃ i mmediately after collection or after storage at ambient temperature for a later use (for example for a second DNA isolation).

Procedure

Lysis

Stool samples are lysed in **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 a pre filled safe look tube and the lysate must only be mixed with them and the matrix with the bound contaminations and cell debris are pelleted by centrifugation an the DNA in the supernatant is pre-cleaned and will be separated from the pellet.

Protein digestion

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

Binding of total DNA

After adding **Binding Buffer P** to the supernatant, the mixture is mixed with magnetic beads and 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

DNA bound to the beads is washed in three steps. 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 buffer from the beads using 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 /KF96 /KFflex 96** procedure from feaces, depends on the healthy status of the donor, the bacteria content, sample source, transport, storage, and age. A typical yield is 10-80 µg, a typical DNA concentration is 50-300 ng/µl. 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.

Important notes

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” (see 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 contaminated gloves
- 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

Preparing reagents and buffers

1. adjust the thermomixer to 70℃.
2. dissolve Proteinase K in dd H_2O,
3. store diluted Proteianse K at –20℃
4. heat heating blocks (e.g. thermomixer) to 70℃ and 95 ℃

96 total DNA extractions:
Add 1,5 ml ddH_2O to **Proteinase K**, mix thoroughly and store the tube at -20℃! Add 80 ml 96-100% ethanol to the 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!
5 x 96 total DNA-extractions:
Add 13 ml ddH_2O to **Proteinase K**, mix thoroughly. Aliquote in 5 parts and store the tubes at -20℃! 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.de)

- microcentrifuge
- thermomixer (for 95℃)
- measuring cylinder (250 ml)
- disposable gloves
- pipet with tips
- reagents reservoirs for multichannel pipets
- 96-100% ethanol
- ddH_2O
- vortexer or other homogenizer

Important indications

1. The kit procedure is also suitable for purifying DNA from very small amounts of starting material. If the sample has 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 the downstream application. In order to prevent any interference of the carrier with the downstream application, a RNA carrier can be used. This can be removed later by RNase digestion. The carrier should be added to the lysis buffer before preparation or to the stabilization buffer – stool mixture, never add to the stool directly.
2. **MAP Solution A** can also purify low amounts of RNA besides DNA. For the elimination of RNA (if necessary) add 20 µl RNase A (10 mg/ ml) before adding the **Binding Buffer P**. Iincubate the sample at room temperature for 5 min. Continue as described in the 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 D** (down to 60 µl). This may result in a higher concentration of DNA. Lower volumes of **Elution Buffer D** will decrease the yield of DNA.
- If low concentrated TRIS-buffer affects sensitive downstream applications, use water for elution. However, ensure that the pH of the water is at least 7.0 (deionized water from certain sources can be acidic). DNA stored in water is subjected to degradation by acid hydrolysis.
- Eluting twice with each 100 µl **Elution Buffer D** is also possible and produces slightly higher yield of DNA. However, a changed elution demands a modification of the assay file within the KF software.

Scheme of the InviMag® Stool DNA Kit /KF96 /KFflex 96

Please read protocols prior the start of the preparation carefully

transfer 200 mg of the stool sample into a 2 ml Safe-Look-Tube (not provided)
add 1,2 ml **Lysis Buffer P** to the sample, vortex for homgenization for 1 min.

for enrichment of host DNA:	incubate 10 min at RT under shaking or
for enrichment of bacterial DNA:	incubate 10 min at 95℃ on a thermomixer under shaking, add 5 **Zirconia Beads II** to the homogenate and vortex for 2 min

During lysis prefill all plates with needed buffer and the appropriate volumes

Tip Plate : place in the KF 96 Tip Comb for DW magnets on a 200 µl **Elution Plate**

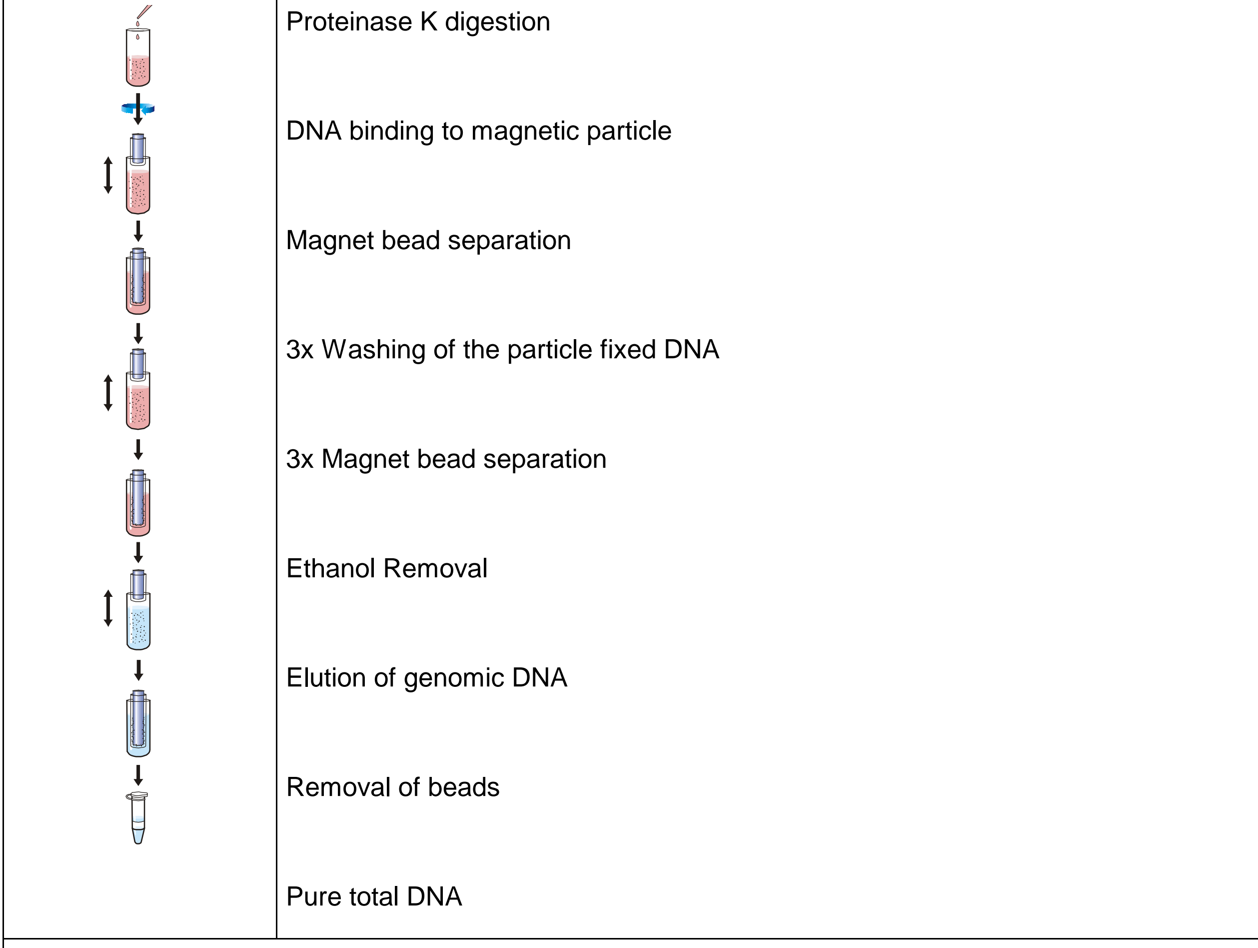
Binding Plate: 25 µl **Proteinase K**

Washing Plate 1: 800 µl **Wash Buffer I**
Washing Plate 2: 800 µl **Wash Buffer II**
Washing Plate 3: 800 µl **Wash Buffer II**
Elution Plate: 200 µl **Elution Buffer D**

spin down at 13.400 x g (12.000 rpm) for 1 min

transfer the supernatant to an **InviAdsorb Tube** and mix it by vortex
incubate 1 min at RT and spin down for 3 min at max. speed

transfer 400 µl of the supernatant into the Binding Plate and add 25 µl **Proteinase K** (if not added yet).
Start the run (for detailed information see chapter “Starting a run on a KF instrument”)



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

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: *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 reaction tube (not provided). Add 1.2 ml **Lysis Buffer P** to each stool sample. Vortex vigorously for 1 min.

Important: *If the sample is liquid, transfer 200 µl into a 2.0 ml reaction tube. Cut the end of the pipet tip to make pipetting easier.If the sample is frozen, use a scalpel or spatula to scrape bits of stool into a 2.0 ml reaction tube on ice. Take care, that the samples do not thaw until Lysis Buffer P is added, otherwise the DNA in the sample may degrade. After addition of the buffer, the following steps can be performed at RT or like recommended.*

For an enrichment of bacterial DNA :

Incubate the sample for 10 min at 95℃ in a thermo mixer under 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 and beads.

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

Note: ***During the sample lysis prefill the plates of the KingFisher 96 with the following Buffers, respectively.***

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 full speed (13000 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 18.

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.

<u>Important:</u> *Please note, that the extracted DNA from stool sample is by the majority from bacterial origin!*

***<u>Important Note</u>*:** *To lyse some special bacteria completely, (like Mycobacteria paratuberculosis or Chlamydia) a special treatment is necessary*

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

Prepare a container with crushed ice

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

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

1. <u>Sample homogenization and prelysis</u>

- Weigh in 200 mg or 200 µl of the stool sample (fresh or frozen) into a 2.0 ml Safe-Lock-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 minutes
- Add 5–10 Zirconia Beads II to the homogenate.
- Reincubate the sample at 95°C for 3 min, followed by a vortexing step for 2 min.
- Centrifuge the sample at 13.200 x g (12.000 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 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 18.

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.

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 page 27)

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 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.

Important Notes:

*The collected sample can be stored at ambient temperature for at least 3 days. The storage under **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 i mmediately 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 the 2.0 ml Safe-Lock Tube.

For an enrichment of host DNA, don' t 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 is not influencing the amount of human DNA

For an enrichment of bacterial DNA :

Incubate the homogenized sample for 10 min at 95°C in a thermomixer under 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 lead to maximize the amount of bacterial DNA, because of a very efficient destruction of the cell wall of e.g. gram+ bacteria.*

3. 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 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 18.

Additional Protocol 4: Post purification of DNA containing inhibitors

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

1. Eluate adjustment

Adjust your eluate to at least 100 µl, for respective dilution take water.

2. Sephadex G50 Slurry

Make a slurry of Sephadex G50 by adding water to Sephadex G50 powder und soaking until the slurry is reaching its final extension. This is dependent on the amount you are producing, it shouldn't take more than 30 min.

3. Adsorbtion of inhibitors

Add 1/3 of your eluate volume of slurry to the eluate. Incubate for 30 min under continuous shaking at room temperature (RT).

4. Removal of slurry

Centrifuge the mixture at 10.000 x *g* (10.500 rpm). Take the supernatant and transfer it to a new reaction tube, it contains you purified DNA.

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

Starting a run on a KingFisher instrument

KF96 / KFflex 96 Plate setup

Note: ***It is important to mix the bottle with MAP Solution A carefully 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**
Washing Plate 1:	Add 800 µl **Wash Buffer I**
Washing Plate 2:	Add 800 µl **Wash Buffer II**
Washing Plate 3:	Add 800 µl **Wash Buffer II**
Elution Plate (200 µl):	Add 200 µl **Elution Buffer D**

- Transfer 400 µl of the **cleared sample** into the Binding Plate containing 25 µl **Proteinase K**.
- Start the by eihter selecting the assay "**InviMAG_Stool_KF96**" or "**InviMag_Stool_KFflex 96**" depending on the instrument you are using.
- Insert the prefilled plates into 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 the user has to add 300 µl Binding Buffer P and 20 µl MAP A Solution to sample containing cavities of the Binding Plate. After addition, reinsert the plate into instrument and continue the assay 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 KF 96 instrument

[PROTOCOL PROPERTIES]

Name = InviMAG_Stool_KF96
Protocol template version = 2.6.0
Instrument type = KingFisher 96
Description = KF96 (Thermo Electron) protocol for Isolation of DNA from stool samples with the InviMAG Stool DNA Kit / KF96 /KFflex 96
Kit = InviMAG Stool DNA Kit / KF96 /KFflex 96

[PLATE LAYOUTS]

Binding plate

Plate type = Thermo DW
Plate change message = Insert Bind plate

A:
- volume = 400, name = Lysed sample
- volume = 25, name = Proteinase K

Washing plate_1

Plate type = Thermo DW
Plate change message = Insert Wash_1

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

Washing plate_2

Plate type = Thermo DW
Plate change message = Insert Wash_2

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

Washing plate_3

Plate type = Thermo DW
Plate change message = Insert Wash_3

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

Elution plate

Plate type = KingFisher 96 plate
Plate change message = Insert Elution

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

Tip plate

Plate type = KingFisher 96 plate
Plate change message = Insert Tip plate

A:
- EMPTY

[STEPS]

HEATING

Step parameters

- Name = Heating
- Plate = Binding plate

Beginning of step:

- Premix = Yes

Heating parameters:

- Heating time = 20min 0s
- Preheat = Yes
- Temperature = 65
- During heating = Mix,
- Postmix time = 0s, speed = Slow

End of step:

- Collect beads = No

PAUSE

Step parameters

- Name = Pause
- Plate = Binding plate
- Message = Add Buffer and Beads
- Dispense:Buffer P and MAP A, volume=320ul

BIND

Step parameters

- Name = Bind
- Plate = Binding plate

Beginning of step:

- Release = Yes, time = 30s, speed = Medium

Bind parameters:

- Bind time = 5min 0s, speed = Slow

End of step:

- Collect beads = Yes, count = 4

WASH1

Step parameters

- Name = Washing 1
- Plate = Washing plate_1

Beginning of step:

- Release = Yes, time = 30s, speed = Fast

Wash parameters:

- Wash time = 2min 0s, speed = Medium

End of step:

- Collect beads = Yes, count = 3

WASH2

Step parameters

- Name = Washing 2
- Plate = Washing plate_2

Beginning of step:

- Release = Yes, time = 30s, speed = Fast

Wash parameters:

- Wash time = 1min 30s, speed = Medium

End of step:

- Collect beads = Yes, count = 3

WASH3

Step parameters

- Name = Washing 3
- Plate = Washing plate_3

Beginning of step:

- Release = Yes, time = 30s, speed = Fast

Wash parameters:

- Wash time = 1min 30s, speed = Medium

End of step:

- Collect beads = Yes, count = 3

DRY

Step parameters

- Name = Dry
- Plate = Washing plate_3
- Dry time = 5min 0s
- Tip position = Outside well

ELUTION

Step parameters

- Name = Elution
- Plate = Elution plate

Beginning of step:

- Release = Yes, time = 30s, speed = Medium

Elution parameters:

- Elution time = 7min 30s, speed = Slow
- Heating = No

Remove beads:

- Remove beads = Yes, collect count = 4,
disposal plate = Washing plate_3

For self programming of the KFflex 96 instrument

[PROTOCOL PROPERTIES]

Name = InviMAG_Stool_KFflex96
Protocol template version = 3.1
Instrument type = KingFisher Flex 96
Description = KFflex 96 (Thermo Electron) protocol for Isolation of DNA from stool samples with the InviMAG Stool DNA Kit /KF96 /KFflex 96
Kit = InviMAG Stool DNA Kit /KF96 /KFflex 96

Layout Data

Tip Plate: KingFisher 96 KF plate

Reagents<empty>

Binding Plate: Microtiter DW 96 plate

Reagents:

NameSample

Volume [µl]400

TypeSample

NameProteinase K

Volume [µl]25

TypeReagent

NameBinding Buffer P [Dispensed]

Volume [µl]300

TypeReagent

NameMAP A Solution [Dispensed]

Volume [µl]20

TypeReagent

Washing Plate 1: Microtiter DW 96 plate

Reagents:

NameWash Buffer I

Volume [µl]800

TypeReagent

Washing Plate 2 Microtiter DW 96 plate

Reagents:

NameWash Buffer II

Volume [µl]800

TypeReagent

Washing Plate 3 Microtiter DW 96 plate

Reagents:

NameWash Buffer II

Volume [µl]800

TypeReagent

Elution Plate: KingFisher 96 KF plate

Reagents:

NameElution Buffer D

Volume [µl]200

TypeReagent

Steps Data

Tip:

Plate: Tip96 DW tip comb

PickUp plate: Tip Plate

Leave plate: Tip Plate

Heating Step

Plate: Binding Plate

Beginning of step:

Precollect: No

Release beads: Yes

Mixing/heating parameters:

Heating temperature [°C]: 65

Preheat: Yes

Mixing time [hh:mm:ss]: 00:20:00

Mixing speed: Medium

End of step:

Postmix: No

Collect beads: No

Pause

Plate: Binding Plate

Message: Add Binding Buffer P + MAP A Solution

Dispensing volume [µl]: 320

Reagent name: Binding Buffer P

Volume [µl]: 300

Reagent name: MAP A Solution

Volume [µl]: 20

Binding

Plate:Binding Plate

Beginning of step:

Precollect:No

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

Release speed: Medium

Mixing/heating parameters:

Heating during mixing: No

Mixing time [hh:mm:ss]: 00:05:00

Mixing speed: Slow

End of step:

Postmix: No

Collect count: 4

Collect time [s]: 3

Washing_1

Plate: Washing Plate 1

Beginning of step:

Precollect: No

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

Release speed: Fast

Mixing/heating parameters:

Heating during mixing: No

Mixing time [hh:mm:ss]: 00:02:00

Mixing speed: Medium

End of step:

Postmix: No

Collect count: 3

Collect time [s]: 3

Washing_2

Plate: Washing Plate 2

Beginning of step:

Precollect: No

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

Release speed: Fast

Mixing/heating parameters:

Heating during mixing: No

Mixing time [hh:mm:ss]: 00:01:30

Mixing speed: Medium

End of step:

Postmix: No

Collect count: 3

Collect time [s]: 3

Washing_3

PlateWashing Plate 3

Beginning of step:

Precollect: No

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

Release speed: Fast

Mixing/heating parameters:

Heating during mixing: No

Mixing time [hh:mm:ss]: 00:01:30

Mixing speed: Medium

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

Drying

PlateWashing Plate 3

Dry time [hh:mm:ss]: 00:05:00
Tip position: Outside well / tube

Elution

Plate: Elution Plate

Beginning of step:
Precollect: No

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

Mixing/heating parameters:
Heating during mixing: No
Mixing time [hh:mm:ss]: 00:07:30
Mixing speed: Slow

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

RemoveBeads

Plate: Washing Plate 3

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

Troubleshooting

Problem	Comments and suggestions
low amount or no DNA of extracted DNA	
sample stored incorrectly	sample should be stored at 4℃ or – 20℃
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 P**	mix sample sufficient by pipetting up and down with **Binding Buffer P** 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 minutes at room temperature before elution
low amount of **MAP Solution A**	mix **MAP Solution A** thoroughly before pipeting 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 P** 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. To maximize PCR specificity, it is recommend the use of **InviTaq Hot Start Taq DNA Polymerase** (see ordering information on page 27).
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℃ and/or add zirconia beads to the stool samples lysis mixture (see **InviMag® Stool DNA 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 "A260/A280 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 P**. 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 14). **See also protocol: Post Purification, see page 19**
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 P** to the eluate, and continue with step 5 of "Protocol: Isolation of total DNA from up to 400 mg stool samples with and without enrichment of bacterial DNA " (page 14).
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	
insufficient centrifugal force	increase the centrifugation time proportionately if your centrifuge cannot provide 13.400 x g (12.000 rpm), e.g. instead of centrifuging for 1 minutes at 13.400 x g, centrifuge for 3 minutes at 10,000 x g).

Little or no supernatant visible after centrifugation step with InviAdsorb matrix insufficient centrifugal force	with some samples, centrifugation to precipitate the **InviAsorb** 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 P	in most cases, this effect comes form big amounts of DNA in the sample. Don't remove this precipitate and follow strictly the protocol.

Appendix

KingFisher Software Version 2.6 and 3.1

The KingFisher Software 2.6.2 was used for the creation of the KingFisher 96 protocols whereas the Software 3.1 was used for creation of KingFisher Flex 96 run files. The user can either transfer the protocol onto the workstation or run the the protocol directly from the software. Be aware that directly run protocols are not stored in the workstation memory.

If you don´t have the correct KingFisher software installed on your computer, please call your local ThermoFisher distributor for an update.

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

Minimal PC Requirements for KingFisher Software 2.6 and 3.1

PC requirements	
Interface	Serial communication port via a RS-232 full duplex interface
Supported operating systems	Microsoft Windows 2000 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 Kit	10 purifications	1438100900
InviMag® Stool DNA Kit	50 purifications	1438100200
InviMag® Stool DNA 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