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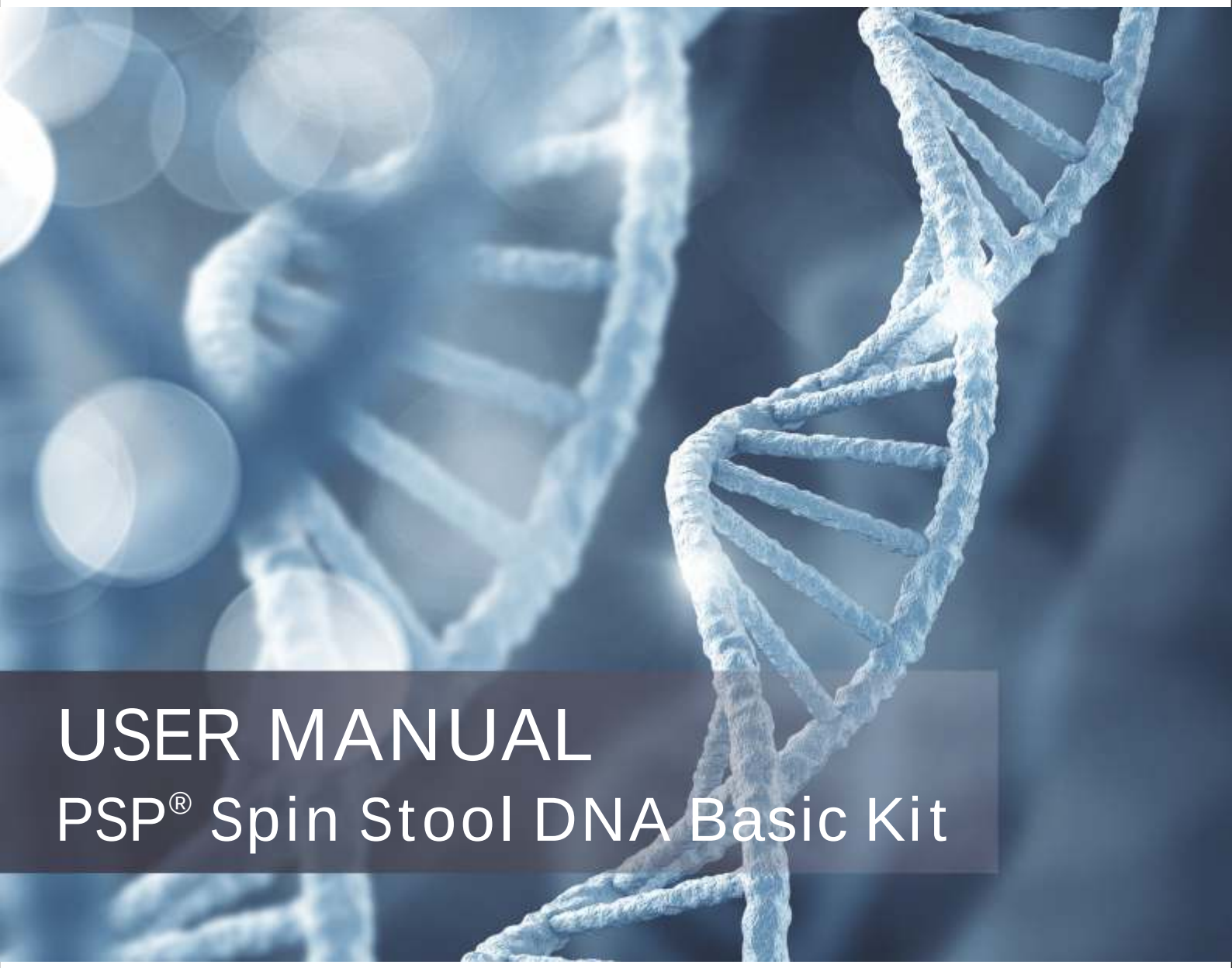

USER MANUAL

PSP® Spin Stool DNA Basic Kit

For purification of total DNA from fresh, frozen or stabilized stool samples
(using in combination with the Stool DNA Stabilizer or the Stool Collection Tubes with Stool DNA Stabilizer)

CE IVD

REF 1038120X00

Invitek Molecular GmbH · D-13125 Berlin

Instruction

PSP® Spin Stool DNA Basic Kit

The **PSP® Spin Stool DNA Basic Kit** is a DNA purification system for stool samples. The kit has been designed for isolation of DNA from microorganisms, as well as for isolation of DNA from the host organism. The purified DNA is ideal for reliable use in PCR and other downstream enzymatic reactions.

The **PSP® Spin Stool DNA Basic Kit** contains all components necessary for sample extraction except a lysis buffer (Stool DNA Stabilizer). Depending on the application the Stool DNA Stabilizer can be added for sample lysis and storage. If a comprehensive sample management is required, the Stool Collection Tubes with DNA Stabilizer are recommended to complement the PSP® Spin Stool DNA Basic Kit.

If the **PSP® Spin Stool DNA Basic Kit** is used together with the **Stool DNA Stabilizer**, the Invisorb® technology allows fast and easy purification of total DNA from up to 200 mg fresh or frozen stool samples. The purified DNA is of high quality and well suited for use in *in-vitro* diagnostics.

The use of the **PSP® Spin Stool DNA Basic Kit** in combination **with Stool Collection Tubes with DNA Stabilizer** provides complete sample management from sample collection, transport and storage to lysis of the sample. The DNA stabilizer guarantees a stable sample storage of up to three months at room temperature, the lysis function preserves the microorganism titre of the sample.

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

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

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Kit content of the PSP® Spin Stool DNA Basic Kit

	50 extractions	250 extractions
Catalog No.	1038120200	1038120300
InviAdsorb	50	5 x 50
Zirconia Beads II	2 vials	8 vials
Proteinase K	for 1.5 ml working solution	for 5 x 1.5 ml working solution
Binding Buffer A	9 ml (final volume 30 ml)	36 ml (final volume 120 ml)
Wash Buffer I	30 ml (final volume 60 ml)	80 ml (final volume 160 ml)
Wash Buffer II	18 ml (final volume 60 ml)	2 x 45 ml (final volume 2 x 150 ml)
Elution Buffer	15 ml	60 ml
2.0 ml Safe-Lock-Tubes	2 x 50	2 x 250
RTA Spin Filter Set	50	5 x 50
RTA Receiver Tubes	2 x 50	10 x 50
1.5 ml Receiver Tubes	2 x 50	10 x 50
Manual	1	1
Initial steps	Add 21 ml 99.7% Isopropanol to the **Binding Buffer A.** Mix by intensive shaking for 1 min. Shortly before use mix by inverting several times. Add 1.5 ml ddH_2O to **Proteinase K,** mix thoroughly until completely dissolving. Add 30 ml of 96 - 100% ethanol to the bottle **Wash Buffer I,** mix thoroughly and always keep the bottle firmly closed! Add 42 ml of 96 – 100 % ethanol to the bottle **Wash Buffer II**, mix thoroughly and always keep the bottle firmly closed! Preheat needed amount of **Elution Buffer** at 70°C in a thermomixer	Add 84 ml 99.7% Isopropanol to the **Binding Buffer A.** Mix by intensive shaking for 1 min. Shortly before use mix by inverting several times. Add 1.5 ml ddH_2O to each tube **Proteinase K**, mix thoroughly until completely dissolving. Add 80 ml of 96 - 100% ethanol to the bottle **Wash Buffer I,** mix thoroughly and always keep the bottle firmly closed! Add 105 ml of 96 - 100% ethanol to each bottle **Wash Buffer II**, mix thoroughly and always keep the bottle firmly closed! Preheat needed amount of **Elution Buffer** at 70°C in a thermomixer

Important: *For stool sample collection, stabilization transport and storage the **Invitek Molecular Stool Collection Tubes with DNA Stabilizer** or the **Stool DNA Stabilizer reagent** should be ordered additionally.*

*We recommend ordering separately the **PSP® Spin Stool DNA Basic Kit** and **Stool Collection Tubes with DNA Stabilizer** if samples are collected at different places or periods (see ordering information at page 27).*

Components for sample lysis and sample management

Stool DNA Stabilizer*)	1038111100	180 ml
Stool Collection Tube with DNA Stabilizer	1038111200	50 tubes
Stool Collection Tube with DNA Stabilizer	1038111300	250 tubes

) For 50 reactions one bottle of Stool DNA Stabilizer is suitable, whereas for 250 reactions two bottles are needed.

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 **PSP® Spin Stool DNA Basic Kit,** except **dissolved Proteinase K** 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).

Stool Collection Tube with DNA Stabilizer: If there are any precipitates in the provided solutions dissolve them by carefully warming them up. Please incubate for 5 min. in a 30°C water bath or in a 37°C incubator. After complete dissolving mix the buffer by gently shaking. The precipitates do not influence the functionality of the buffer.

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. Like this, the dissolved Proteinase K is stable as indicated on the kit package.

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

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

Quality control and product warranty

Invitek Molecular guarantees the full functionality of the **PSP® Spin Stool DNA Basic Kit** for applications described in this manual. The user must examine the suitability of the product for its particular use. If a product does not meet the requirements for an application described in this manual, Invitek Molecular will check the lot number of the product. If a problem is identified by the manufacturer, the product will be replaced 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 **PSP® Spin Stool DNA Basic Kit** have been tested against predetermined specifications.

If you have any questions or problems regarding any aspects of the **PSP® Spin Stool DNA Basic Kit** 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 available at the Invitek Molecular webpage.

For technical support or further information, please contact:
Email: techsupport@invitek-molecular.com or contact your local distributor.

Intended use

The **PSP® Spin Stool DNA Basic Kit** in combination with the **Stool DNA Stabilizer** or the **Stool Collection Tubes with DNA Stabilizer** can be used for isolation of DNA from microorganisms, as well as for isolation of DNA from host organisms.

Before using the kit, please ensure that you have read the instructions and are fully informed about the purpose and limitations of use of the kit (see "Product use limitation", and "Features of the PSP® Spin Stool DNA Basic Kit").

The protocols for the isolation and all buffers are optimized for a high yield as well as a high purity. All hands on steps are reduced to a minimum.

Any diagnostic results generated using the sample preparation procedure in conjunction with any downstream diagnostic assays should be interpreted with regard to other clinical or laboratory finding.

THE PRODUCT IS INTENDED 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 other enzymatic modifications of DNA followed by signal detection or amplification. Any diagnostic results generated by using the sample preparation procedure in conjunction with any downstream diagnostic assay should be interpreted with regard to other clinical or laboratory findings.

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

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

Product use limitation

The kit is not validated for the isolation of genomic DNA from cultured or isolated cells, from tissue, swabs, dried blood stains, or cell free body fluid, like synovial fluid and urine, or the purification of RNA. The application of the kit for isolation and purification of viral DNA has not been evaluated.

The included chemicals can only be used once. Any deviation of the source material or the flow line can lead to inoperability; therefore, in this case, no warranty or guarantee is given, either implicitly or explicitly**.** Invitek Molecular will be released of its responsibilities if other sample materials than described are processed or if the sample preparation protocols are changed or modified.

The user is responsible to validate the performance of the Invitek Molecular product for any particular use. Invitek Molecular does not provide validation of the performance characteristics of the product in relation 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 according 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 it is guaranteed that they work as described here. Any problems, incidents or defects shall be reported to Invitek Molecular immediately upon detection.

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

The product is not suitable for consumption.

Safety information

When and while working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles and avoid skin contact. Adhere to the legal requirements for working with biological material!

For more information, please consult the appropriate material safety data sheets (MSDS). These are available online in PDF format at www.invitek-molecular.com for all Invitek Molecular products and their 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 **PSP® Spin Stool DNA Basic Kit** procedures for residual infectious materials. Contamination of the liquid waste with residual infectious materials is very unlikely, but cannot be excluded completely. Therefore, liquid waste must be considered infectious and must be handled and discarded according to local safety regulations.

European Community risk and safety phrases for the components of the **PSP® Spin Stool DNA Basic Kit** and **Stool DNA Stabilizer** are listed as follows:

Stool DNA Stabilizer

Warning
H319 –H412.-P280- P305-351-338-P273

Proteinase K

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

Wash Buffer I

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

H302: Harmful if swallowed.
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.
H412: Harmful to aquatic life with long lasting effects.
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
in USA: 1 – 800 – 535 – 5053

Features of the PSP® Spin Stool DNA Basic Kit

Stool samples typically contain many compounds that can degrade DNA and inhibit downstream enzymatic reactions. To ensure the removal of these contaminants the **PSP® Spin Stool DNA Basic Kit** contains tubes with **InviAdsorb** and optimized essential washing conditions to remove all potent inhibitors very efficiently. Therefore, the simple PSP® Spin procedure yields pure DNA ready-to-use in less than 1h.

A rigorous prelysis step using **Zirconia Beads II** with optimized prelysis buffer under high temperatures is followed by a 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 also from Gram-positive 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 choice of different lysis conditions allows the enrichment or a reduction of the content of bacterial DNA in the total DNA. All impurities are removed very efficiently during washing steps and the purified DNA is eluted directly in a low-salt buffer.

No phenol/chloroform extraction, ethanol precipitation or ß-Mercaptoethanol are used. 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°C for later use.

Due to the high purity, the isolated total DNA is suitable for a broad range of downstream applications (see below) or can be stored at -20°C for subsequent use.

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

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 purify high molecular weight total DNA using magnetic beads, Invitek Molecular offers different kits for use on the KingFisher™ workstations.

Features of the PSP® Spin Stool DNA Basic Kit in combination with the Stool DNA Stabilizer

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

The **PSP® Spin Stool DNA Basic Kit** allows in combination with the Stool DNA Stabilizer rapid and efficient isolation of high quality DNA from up to 200 mg fresh or frozen stool sample.

Features of the PSP® Spin Stool DNA–Basic Kit in combination with Stool Collection Tubes with DNA Stabilizer

Starting material	Yield	Time	Ratio
1.4 ml Stool DNA Stabilizer with stool homogenate	up to 50 µg (depends on starting material)	about 45 min (incl. lysis time)	A_{260} : A_{280} 1.4 – 1.8

The **PSP® Spin Stool DNA Basic Kit** in combination with **Stool Collection Tubes with DNA Stabilizer** allows the collection and storage of stool samples as well as the stabilization of the stool specimen without any degradation of the DNA with a very efficient and fast isolation of high quality total DNA.
The **Stool DNA Stabilizer** enables the storage of the stool samples after collection without cooling under ambient temperature for at least 3 months.

The **Stool DNA Stabilizer** preserves the microorganism titer by lyses of gram-positive and –negative bacteria and inactivation of DNases. For the DNA extraction process only a small amount of the total volume will be used, the residual sample can be used for further extractions or a long-term storage at -20°C.

Principle and procedure

The **PSP® Spin Stool DNA Basic Kit** procedure comprises the following steps:

- Lysis of sample
- Removal of PCR inhibitors
- Protein digestion
- Binding the nucleic acids to the membrane of a spin column
- Washing of the spin column and hereby elimination of contaminants and ethanol
- Elution of the nucleic acids

After homogenization of the sample with **Stool DNA Stabilizer** human cells and the bacterial cell wall will be lysed differently (depending on the temperature profile). The lysate will be mixed with **InviAdsorb** and most of the PCR inhibiting components will be removed followed by a protein digestion. After lysis the DNA binds to the membrane, contaminations and enzyme inhibitors are efficiently removed during the following three washing steps, and highly purified DNA is eluted in **Elution Buffer** or water.

The **PSP® Spin Stool DNA Basic Kit** in combination with the Stool Collection Tube with DNA Stabilizer comprises additional steps:

- Sample collection, storage and transport
- DNA stabilization in the sample

The DNA in the stabilized sample is stable for more than 3 months at RT and can be transported to the lab without degradation.

Sampling and storage of starting material

Stool DNA Stabilizer

The collected fresh stool sample can be stored for at least 1-2 hours at RT. Because of the high content of DNases, which lead to rapid digestion and degradation of DNA, the Stool DNA Stabilizer should be added to the sample as soon as possible. Otherwise, the non-stabilized stool sample can be stored at -20°C for a certain time.

Stool Collection Tubes with DNA Stabilizer

The storage of fresh samples in **DNA Stabilizer** provided in the Stool Collection Tubes, allows storage at RT for about 3 months. The storage of fresh samples in **DNA Stabilizer** will lead to less degraded DNA and a better yield of bacterial pathogens with difficult to lyse cell walls. Storage time below 3 months has no influence on the quality or the amount of host cell DNA. The collected sample in Stool DNA Stabilizer can also be used immediately for the isolation of DNA after collection.

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).

Procedure

Lysis
Stool samples are lysed in **Stool DNA Stabilizer** under denaturing conditions at high 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°C. A heating step is recommended for cells that are difficult to lyse (e.g. Gram-positive bacteria).

Note: *The total DNA concentration in the lysate is increased 3-5 fold by lysis at 95°C, also the ratio of non-human to human DNA is increased.*

Removal of PCR inhibitors
After lysis, DNA damaging substances and PCR inhibitors, which are present in the feces are adsorbed efficiently to the **InviAdsorb** matrix. **InviAdsorb** is provided in safe lock tubes and the lysate must only be mixed with the matrix. The bound contaminants and cell debris are pelleted by centrifugation. The supernatant contains the pre-cleaned DNA.

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

Binding of total DNA
After adding **Binding Buffer A** to the supernatant, the mixture is transferred to a spin column and nucleic acids are bound to the membrane of the RTA Spin Filter during a brief centrifugation step.
Optimal salt concentrations and pH conditions in the lysate ensure that residues of digested proteins and other contaminations, which can inhibit downstream enzymatic reactions, are not retained on the Invisorb® membrane.

Removing residual contaminants
DNA bound to the Invisorb® membrane is washed in two centrifugation steps. Contaminants are efficiently and completely removed using **Wash Buffer I** and **II**, while the nucleic acids remain bound to the membrane.

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

Yield and quality of total DNA

The amount of purified DNA from feces depends on the health status of the donor, bacteria content, sample source, transport, storage, and age. A typical yield is 10 – 60 µg, a typical DNA concentration is 50 – 600 ng/ µl. Yield and quality of isolated genomic DNA is suitable for any molecular diagnostic detection system. Diagnostic tests should be performed according to manufacturers' instructions.

Important notes

Important points before starting a protocol

Check the product and its components as well as the packaging for obvious damage, correct quantities and quality immediately upon receipt of the product. If there are any deviations, please notify Invitek Molecular in writing with immediate effect after examination. If bottles containing buffer are damaged, contact the Invitek Molecular Technical Services or your local distributor. In case of liquid spillage, refer to "Safety Information" (see page 7). Do not use damaged kit components, since their use may lead to poor kit performance.

- Always change pipet tips between liquid transfers. To avoid cross-contamination, the use of filter tips is recommended.
- All centrifugation steps are carried out at room temperature.

- When working with chemicals, always wear a lab coat, disposable gloves, and protective goggles.
- Discard gloves if they become contaminated.
- Do not mix kit components with components from other 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 personnel trained in vitro diagnostic laboratory practice.

Preparing reagents and buffers

1. Adjust the thermomixer to 70°C.
2. Dissolve Proteinase K in ddH_2O.
3. Warm up the needed amount of **Elution Buffer** to 70°C, (100 - 200 µl **Elution Buffer** are needed per sample).
4. Set heating blocks (e.g. thermomixer) to 70°C and 95 °C.
5. Label the needed amount of 2.0 ml RTA Spin Filter Sets.
6. Label the needed amount of 1.5 ml Receiver Tubes (per sample: 1 Receiver Tube), add the needed amount of ethanol to the **Wash Buffer I** and **II** (see Kit contents, page 3).

50 total DNA extractions:
Add 21 ml 99.7% Isopropanol to the **Binding Buffer A.** Mix by intensive shaking for 1 min. Shortly before use mix by inverting several times. Add 1.5 ml ddH_2O to the vial **Proteinase K**, mix thoroughly until completely dissolving. Add 30 ml 96-100% ethanol to the bottle **Wash Buffer I.** Add 42 ml 96-100% ethanol to each bottle **Wash Buffer II.** Mix thoroughly and always keep the bottle firmly closed.
250 total DNA extractions:
Add 84 ml 99.7% Isopropanol to the **Binding Buffer A.** Mix by intensive shaking for 1 min. Shortly before use mix by inverting several times. Add 1.5 ml ddH_2O to each vial **Proteinase K**, mix thoroughly until completely dissolving. Add 80 ml 96-100% ethanol to each bottle **Wash Buffer I.** Add 105 ml 96-100% ethanol to each bottle **Wash Buffer II.** Mix thoroughly and always keep the bottle firmly closed.

Equipment and reagents to be provided 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). (Download as PDF: www.invitek-molecular.com)

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

*) The **PSP® Spin Stool DNA Basic Kit** 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 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 genomic DNA) 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–that can later be removed by RNase digestion. The carrier should be added to the lysis buffer before preparation or to the stabilization buffer, never add to the stool directly.
2. Invisorb® RTA Spin filter 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 A**. Vortex briefly and incubate the sample at room temperature for 5 minutes. Then go on as described in the protocol.

Elution of DNA

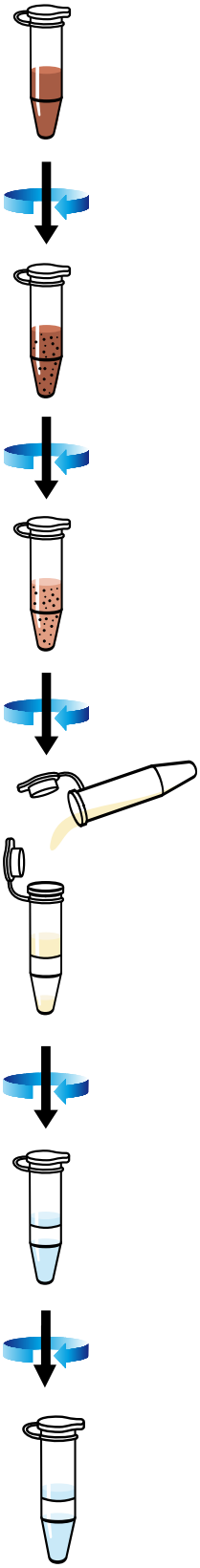
- For downstream applications, that require small starting volumes, a more concentrated eluate may increase assay sensitivity. The elution can be done by using a lower volume of **Elution Buffer** (down to 50 µl). This may result in a higher concentration of DNA. But lower volumes of **Elution Buffer** will decrease the yield of DNA.
- The final volume of eluate recovered can be up to 5 µl less than the volume of elution buffer applied to the spin filter.
- If low concentrated TRIS-buffer affects sensitive downstream applications, use distilled sterile 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 can be degraded by acid hydrolysis.
- Eluting twice with each 100 µl **Elution Buffer** is also possible and gives slightly higher yield of DNA.

Handling of the RTA Spin Filter Set

Due to the sensitivity of DNA amplification technologies, the following precautions are necessary:

- to avoid cross-contamination between sample preparation when handling RTA Spin Filter Set apply the sample or solution to the RTA Spin Filter Set carefully, pipet the sample into the filter without wetting the rim of the column
- always change pipet tips between liquid transfers, the use of filter tips is recommended
- avoid touching the RTA Spin Filter membrane with the pipet tip

Scheme of the PSP® Spin Stool DNA Basic Kit in combination with the Stool DNA Stabilizer



Please read protocols carefully prior starting the preparation procedure.

Transfer 200 mg of the stool sample into a 2 ml Safe-Lock-Tube.
Add 1.2 ml **Stool DNA Stabilizer,** vortex for 1 min.

For enrichment of host DNA:
Incubate 10 min at RT under shaking.

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

Spin down at 11.100 x g (11.000 rpm) for 1 min.

Transfer the supernatant to the InviAdsorb-Tube.
Mix it by vortexing for 15 sec.
Incubate 1 min at RT.
Spin down for 3 min at full speed.

Transfer the supernatant in a new 1.5 ml Receiver Tube.
Centrifuge the sample again at full speed for 3 min.

Add 25 µl **Proteinase K** to a new 2.0 ml Safe-Lock-Tube.
Transfer 400 µl of the supernatant to the 2.0 ml Safe-Lock-Tube with Proteinase K.

Mix shortly by vortexing.
Incubate for 10 min at 70 °C while continuously shaking on a thermomixer at 900 rpm.

Add 200 µl **Binding Buffer A** *(follow preparing instructions)* to the lysate.
Mix shortly by vortexing or pipetting up and down.

Transfer the whole mixture to the RTA Spin Filter.
Incubate for 1 min at RT.
Centrifuge at 11.000 x g (11.000 rpm) for 2 min.
Discard the filtrate and the RTA Receiver Tube.

Transfer the RTA Spin Filter in a new RTA Receiver Tube.
Pipet 500 µl **Wash Buffer I** onto the RTA-Spin Filter.
Centrifuge at 11.000 x g (11.000 rpm) for 1 min.
Discard the flow-through and the RTA Receiver Tube.

Put the RTA Spin Filter in a new RTA Receiver Tube.
Pipet 700 µl **Wash Buffer II** onto the RTA Spin Filter.
Centrifuge at 11.000 x g (11.000 rpm) for 1 min.
Discard the flow-through and reuse the RTA Receiver Tube.

To eliminate any traces of ethanol, centrifuge again for 4 min at maximum speed, discard the RTA Receiver Tube.

Transfer the RTA Spin Filter into a new 1.5 ml Receiver Tube.
Pipet 100 - 200 µl of **Elution Buffer** (preheated to 70°C) directly onto the center of the membrane of the RTA Spin Filter.
Incubate for 1 min at RT.
Centrifuge at 11.000 x g (11.000 rpm) for 1 min.

Discard the RTA-Spin Filter.
Place the eluted total DNA immediately on ice and store at –20°C.

Instructions

The note is valid for all protocols:

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

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

Please read the instructions carefully and carry out preparatory arrangements in advance.

Attention: *Please be aware, that you have to prepare the **Binding Buffer A** – see instruction page: 12*

***Important Note*:** *Please note that the majority of extracted DNA from stool samples is of bacterial origin!*

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

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

1. Sample homogenization and prelysis

Weigh 200 mg of stool sample (fresh or frozen) into a 2.0 ml Safe-Lock-Tube and add 1.2 ml **Stool DNA Stabilizer** to each stool sample. Vortex vigorously for 1 min. Even if you use less starting material, perform the protocol as described**.**

Important: *If the sample is liquid, pipet 200 µl into the 2.0 ml Safe-Lock-Tube. Cut-off 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 the provided 2.0 ml Safe-Lock-Tube on ice. Take care that samples do not thaw until Stool DNA Stabilizer is added, otherwise the DNA in the sample may degrade.

Incubate the sample for 10 min at RT under continuous shaking at 900 rpm.
Centrifuge the sample at 11.000 x g (11.000 rpm) for 1 min to pellet solid stool particles.

For an enrichment of bacterial DNA:

Incubate the sample for 10 min at 95°C in a thermomixer under continuous shaking at 900 rpm.
Add 5 **Zirconia Beads II** to the homogenate and vortex for 2 min at RT.
Centrifuge the sample at 11.000 x g (11.000 rpm) for 1 min to pellet solid stool particles and beads.

Important: *The incubation step at 95°C will maximize the yield of bacterial DNA, because of a very efficient disruption of the cell wall of e.g. Gram-positive bacteria.*

For an enrichment of host DNA, do not perform this high-temperature step!

2. Removal of PCR inhibitors

Transfer the supernatant into an **InviAdsorb-Tube** and vortex vigorously for 15 sec.
Incubate the suspension for 1 min at room temperature. Centrifuge the sample at full speed for 3 min.

3. Second sample cleanup

Transfer the supernatant completely into a new 1.5 ml Receiver Tube. Discard the pellet. Centrifuge the sample again at full speed for 3 min.

4. Proteinase K digestion

Transfer 25 µl **Proteinase K** into a new 2.0 ml Safe-Lock-Tube and pipet 400 µl of the supernatant from step 3 to the 1.5 ml Receiver Tube containing **Proteinase K**. Mix shortly by vortexing and incubate the sample for 10 min at 70°C in a thermomixer under continuous shaking at 900 rpm.

5. Binding of the DNA

Add 200 µl **Binding Buffer A** to the lysate and mix shortly by vortexing or by pipetting up and down several times.
Transfer the mixture completely onto the membrane of the RTA Spin Filter. Incubate for 1 min at room temperature and centrifuge at 11.000 x g (11.000 rpm) for 2 min. Discard the filtrate and the RTA Receiver Tube.

6. Washing steps

Put the RTA Spin Filter in a new RTA Receiver Tube. Add 500 µl **Wash Buffer I** to the membrane of the RTA Spin Filter. Close the lid and centrifuge at 11.000 x g (11.000 rpm) for 1 min. Discard the filtrate and the RTA Receiver Tube.

Put the RTA Spin Filter in a new RTA Receiver Tube. Add 700 µl **Wash Buffer II** to the membrane of the RTA Spin Filter. Close the lid and centrifuge at 11.000 x g (11.000 rpm) for 1 min. Discard the filtrate and put the RTA Spin Filter back to the same RTA Receiver Tube.

7. Ethanol removal

To eliminate any traces of ethanol, centrifuge again for 4 min at maximum speed, discard the RTA Receiver Tube.

8. DNA elution

Place the RTA Spin Filter into a new 1.5 ml Receiver Tube and add 100 - 200 µl preheated (70°C) **Elution Buffer** to the sample**.** Incubate for 1 min at RT. Centrifuge at 11.000 x g (11.000 rpm) for 1 min. to elute the DNA. Finally discard the RTA Spin Filter.

Note: *The DNA can also be eluted with a lower volume of Elution Buffer (depends on the expected yield of genomic DNA). But pay attention that the minimum volume for the elution is* ***50 µl and that this volume can reduce the maximum yield.*** *If a quite large amount of DNA is expected, the volume of elution can be increased.*

Note: *For long-term storage, we recommend to keep the eluted DNA at –20°C.*

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

Please read the instructions carefully and carry out preparatory arrangements in advance.

Attention: *Please be aware, that you have to prepare the **Binding Buffer A** – see instruction page: 12*

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

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

Prepare a container with crushed ice.

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

1. Sample homogenization and prelysis

Weigh 200 mg of stool sample (fresh or frozen) into a 2.0 ml Safe-Lock-Tube and add 1.2 ml **Stool DNA Stabilizer** to each stool sample. Vortex vigorously for 1 min.

Important: *If the sample is liquid, pipet 200 µl into the 2.0 ml Safe-Lock-Tube. Cut the end of the pipet tip to make pipetting easier.*

Incubate the homogenized sample for 10 min at 95°C in a thermomixer under continuous shaking at 900 rpm.

Incubate the sample on ice for 3 minutes. Add 5 **Zirconia Beads II** to the homogenate

Put the sample back to the 95°C thermoblock. Incubate for further 3 min at 95°C.

Vortex the sample for 2 min. Centrifuge the sample at 11.000 x g (11.000 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 sec.
Incubate the suspension for 1 min at room temperature. Centrifuge the sample at full speed for 3 min.

3. Second sample cleanup

Transfer the supernatant completely into a new 1.5 ml Receiver Tube. Discard the pellet.
Centrifuge the sample again at full speed for 3 min.

4. Proteinase K digestion

Transfer 25 µl **Proteinase K** into a new 2.0 ml Safe-Lock-Tube and pipet 400 µl of the supernatant from step 3 to the 1.5 ml Receiver Tube containing **Proteinase K**, mix shortly by vortexing and incubate the sample for 10 min at 70°C in a thermomixer under continuous shaking at 900 rpm.

5. Binding of the DNA

Add 200 µl of **Binding Buffer A** to the lysate and mix shortly by vortexing or by pipetting up and down several times.
Transfer the mixture completely onto the membrane of the RTA Spin Filter. Incubate for 1 min at room temperature and centrifuge at 11.000 x g (11.000 rpm) for 2 min. Discard the filtrate and the RTA Receiver Tube.

6. Washing steps

Put the RTA Spin Filter in a new RTA Receiver Tube. Add 500 µl **Wash Buffer I** to the membrane of the RTA Spin Filter. Close the lid and centrifuge at 11.000 x g (11.000 rpm) for 1 min. Discard the filtrate and the RTA Receiver Tube.

Put the RTA Spin Filter in a new RTA Receiver Tube. Add 700 µl **Wash Buffer II** to the membrane of the RTA Spin Filter. Close the lid and centrifuge at 11.000 x g (11.000 rpm) for 1 min. Discard the filtrate and put the RTA Spin Filter back to the same RTA Receiver Tube.

7. Ethanol removal

To eliminate any traces of ethanol, centrifuge again for 4 min at maximum speed, discard the RTA Receiver Tube.

8. DNA elution

Place the RTA Spin Filter into a new 1.5 ml Receiver Tube and add 100 - 200 µl preheated (70°C) **Elution Buffer.** Incubate for 1 min. Centrifuge at 11.000 x g (11.000 rpm) for 1 min. to elute the DNA. Finally discard the RTA Spin Filter.

Note: *The DNA can also be eluted with a lower volume of Elution Buffer (depends on the expected yield of genomic DNA). But pay attention that the minimum volume for the elution is* ***50 µl and that this volume can reduce the maximum yield.*** *If a quite large amount of DNA is expected, the volume of elution can be increased.*

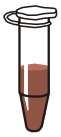
Note: *For long-term storage, we recommend to keep the eluted DNA at –20°C.*

Scheme of the PSP® Spin Stool DNA Basic Kit in combination with Stool Collection Tubes with DNA Stabilizer

Please read protocols carefully prior starting the preparation procedure.

Collect a spoon of the stool sample.
Transfer stool sample into the **Stool Collection Tube with DNA Stabilizer**, close the tube.
Mix thoroughly by shaking or vortexing to dissolve the sample.

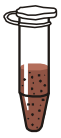
Transfer 1.4 ml of the stabilized stool sample (Stool DNA Stabilizer with stool specimen) into a 2.0 ml Safe-Lock Tube.



For enrichment of host DNA:
Incubate 10 min at RT under shaking.

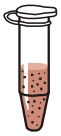
For enrichment of bacterial DNA:
Incubate 10 min at 95°C on a thermomixer under shaking,
add 5 **Zirconia Beads II** to the homogenate and vortex for 2 min.

Spin down at 11.000 x g (11.000 rpm) for 1 min.



Transfer the supernatant to the **InviAdsorb-**Tube.
Mix it by vortexing for 15 sec.
Incubate 1 min at RT.
Spin down for 3 min at full speed.

Transfer the supernatant in a new 1.5 ml Receiver Tube.
Centrifuge the sample again at full speed for 3 min.

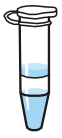


Add 25 µl **Proteinase K** in a new 2.0 ml Safe-Lock-Tube.
Transfer 800 µl of the supernatant to the same tube.
Mix shortly by vortexing.
Incubate for 10 min at 70 °C while continuously shaking on a thermomixer at 900 rpm.

Add 400 µl **Binding Buffer A** *(follow preparing instructions)* to the lysate.
Mix shortly by vortexing or pipetting up and down.

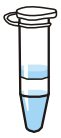
Transfer the whole mixture **in two steps** to the RTA Spin Filter.
Incubate for 1 min at RT.
Centrifuge at 11.000 x g (11.000 rpm) for 2 min.
Discard the filtrate after each centrifugation and the RTA Receiver Tube at the end.

Transfer the RTA Spin Filter in a new RTA Receiver Tube.
Pipet 500 µl **Wash Buffer I** onto the RTA-Spin Filter.
Centrifuge at 11.000 x g (11.000 rpm) for 1 min.
Discard the flow-through and the RTA Receiver Tube.



Put the RTA Spin Filter in a new RTA Receiver Tube.
Pipet 700 µl **Wash Buffer II** onto the RTA Spin Filter.
Centrifuge at 11.000 x g (11.000 rpm) for 1 min.
Discard the flow-through and reuse the RTA Receiver Tube.

To eliminate any traces of ethanol, centrifuge again for 4 min at maximum speed, discard the RTA Receiver Tube.



Transfer the RTA Spin Filter into a new 1.5 ml Receiver Tube.
Pipet 100-200 µl of **Elution Buffer** (preheated to 70°C) directly onto the center of the membrane of the RTA Spin Filter.
Incubate for 1 min at RT.
Centrifuge at 11.000 x g (11.000 rpm) for 1 min, discard the RTA-Spin Filter, place the eluted total DNA immediately in a refrigerator or store it at –20°C.

Protocol 3: Collection of the stool sample and stabilization

Please read the instructions carefully and carry out preparatory arrangements in advance.

1. Open the **Stool Collection Tube with DNA Stabilizer** and collect a spoon of the stool sample.
2. Transfer the spoon with the stool sample back into the **Stool Collection Tube with DNA Stabilizer** and close the tube very tight.
3. Mix thoroughly by shaking or vortexing to dissolve the sample. 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 **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 frozen at –20°C immediately after collection or after storage at ambient temperature for later use (for example for a second DNA isolation).

Protocol 4: Isolation of total DNA from 1.4 ml stabilized stool homogenate with and without enrichment of bacterial DNA

Please read the instructions carefully and carry out preparatory arrangements in advance.

Attention: *Please be aware, that you have to prepare the **Binding Buffer A** – see instruction page: 12*

Important Note: *Please note that the extracted DNA from stool sample is mostly of bacterial origin!*
Set heating blocks (e.g. thermomixer) to 70°C and 95 °C.
*Preheat the **Elution Buffer** to 70°C (e.g. transfer the needed volume into a tube and place it at the appropriate temperature into a thermomixer).*

1. Sample homogenization and prelysis

Transfer 1.4 ml of the well homogenized stool sample (Stool DNA Stabilizer with stool specimen) after storage or directly after collection into the 2.0 ml Safe-Lock Tube.

Centrifuge the sample at 11.000 x g (11.000 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 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 11.000 x g (11.000 rpm) for 1 min to pellet solid stool particles and beads.

Important Note: *The incubation step at 95°C leads to a maximization of the amount of bacterial DNA, because the cell wall of e.g. gram-positive bacteria is destroyed very efficiently.*

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

2. Removal of PCR inhibitors

Transfer the supernatant into an **InviAdsorb-Tube** and vortex vigorously for 15 sec.
Incubate the suspension for 1 min at room temperature. Centrifuge the sample at full speed for 3 min.

3. Second sample cleanup

Transfer the supernatant completely into a new 1.5 ml Receiver Tube. Discard the pellet.
Centrifuge the sample again at full speed for 3 min.

4. Proteinase K digestion

Transfer 25 µl **Proteinase K** into a new 2.0 ml Safe-Lock-Tube and pipet 800 µl of the supernatant from step 3 to the 1.5 ml Receiver Tube containing **Proteinase K**, mix shortly by vortexing and incubate the sample for 10 min at 70°C in a thermomixer under continuous shaking at 900 rpm.

5. Binding of the DNA

Add 400 µl of **Binding Buffer A** to the lysate and mix shortly by vortexing or by pipetting up and down several times.
Transfer the mixture **in two steps** onto the membrane of the RTA Spin Filter. Incubate for 1 min at room temperature and centrifuge at 11.000 x g (11.000 rpm) for 2 min. Discard the filtrate after each centrifugation and the RTA Receiver Tube at the end.

6. Washing steps

Put the RTA Spin Filter in a new RTA Receiver Tube. Add 500 µl **Wash Buffer I** to the membrane of the RTA Spin Filter. Close the lid and centrifuge at 11.000 x g (11.000 rpm) for 1 min. Discard the filtrate and the RTA Receiver Tube.

Put the RTA Spin Filter in a new RTA Receiver Tube. Add 700 µl **Wash Buffer II** to the membrane of the RTA Spin Filter. Close the lid and centrifuge at 11.000 x g (11.000 rpm) for 1 min. Discard the filtrate and put the RTA Spin Filter back to the same RTA Receiver Tube.

7. Ethanol removal

To eliminate any traces of ethanol, centrifuge again for 4 min at maximum speed, discard the RTA Receiver Tube.

8. DNA elution

Place the RTA Spin Filter into a new 1.5 ml Receiver Tube and add 100 - 200 µl preheated (70°C) **Elution Buffer.** Incubate for 1 min. Centrifuge at 11.000 x g (11.000 rpm) for 1 min to elute the DNA. Finally discard the RTA Spin Filter.

Note: *The DNA can also be eluted with a lower volume of Elution Buffer (depends on the expected yield of genomic DNA). But pay attention that the minimum volume for the elution is **50 µl and that this volume can reduce the maximum yield.** If a quite large amount of DNA is expected, the volume of elution can be increased.*

Note: *For long-term storage, we recommend to keep the eluted DNA at –20°C.*

Protocol 5: Isolation of total DNA from 1.4 ml stabilized stool homogenate from difficult to lyse bacteria

Please read the instructions carefully and carry out preparatory arrangements in advance.

Attention: *Please be aware, that you have to prepare the **Binding Buffer A** – see instruction page: 12*

***Important Note*:** *To lyse some special bacteria completely, (like Mycobacteria paratuberculosis or Chlamydia) a special treatment is needed.*

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

Prepare a container with crashed ice.

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

1. Sample homogenization and prelysis

Transfer 1.4 ml of the well homogenized stool sample (**Stool DNA Stabilizer** with stool specimen) after storage or directly after collection into the 2.0 ml Safe-Lock Tube.

Incubate the homogenized sample for 10 min at 95°C in a thermomixer under continuous shaking at 900 rpm.

Incubate the sample on ice for 3 minutes and put the sample back to the 95°C thermo block, Incubate for further 3 min at 95°C.

Add 5 **Zirconia Beads II** to the homogenate and vortex for 2 min at RT.

Centrifuge the sample at 11.000 x g (11.000 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 sec.
Incubate the suspension for 1 min at room temperature. Centrifuge the sample at full speed for 3 min.

3. Second sample cleanup

Transfer the supernatant completely into a new 1.5 ml Receiver Tube. Discard the pellet.
Centrifuge the sample again at full speed for 3 min.

4. Proteinase K digestion

Transfer 25 µl **Proteinase K** into a new 2.0 ml Safe-Lock-Tube and pipet 800 µl of the supernatant from step 3 to the 1.5 ml Receiver Tube containing **Proteinase K**, mix shortly by vortexing and incubate the sample for 10 min at 70°C in a thermomixer under continuous shaking at 900 rpm.

5. Binding of the DNA

Add 400 µl of **Binding Buffer A** to the lysate and mix shortly by vortexing or by pipetting up and down several times.
Transfer the mixture **in two steps** onto the membrane of the RTA Spin Filter. Incubate for 1 min at room temperature and centrifuge at 11.000 x g (11.000 rpm) for 2 min. Discard the filtrate after each centrifugation and the RTA Receiver Tube at the end.

6. Washing steps

Put the RTA Spin Filter in a new RTA Receiver Tube. Add 500 µl **Wash Buffer I** to the membrane of the RTA Spin Filter. Close lid and centrifuge at 11.000 x g (11.000 rpm) for 1 min. Discard the filtrate and the RTA Receiver Tube.

Put the RTA Spin Filter in a new RTA Receiver Tube. Add 700 µl **Wash Buffer II** to the membrane of the RTA Spin Filter. Close the lid and centrifuge at 11.000 x g (11.000 rpm) for 1 min. Discard the filtrate and put the RTA Spin Filter back to the same RTA Receiver Tube.

7. Ethanol removal

To eliminate any traces of ethanol, centrifuge again for 4 min at maximum speed, discard the RTA Receiver Tube

8. DNA elution

Place the RTA Spin Filter into a new 1.5 ml Receiver Tube and add 100 - 200 µl preheated (70°C) **Elution Buffer.** Incubate for 1 min. Centrifuge at 11.000 x g (11.000 rpm) for 1 min to elute the DNA. Finally discard the RTA Spin Filter.

Note: *The DNA can also be eluted with a lower volume of Elution Buffer (depends on the expected yield of genomic DNA). But pay attention that the minimum volume for the elution is **50 µl and that this volume can reduce the maximum yield.** If a quite large amount of DNA is expected, the volume of elution can be increased.*

Note: *For long-term storage, we recommend to keep the eluted DNA at –20°C.*

Troubleshooting

Problem	Comments and suggestions
Clogged RTA Spin Filter	
Insufficient lysis and/ or too much starting material	Increase lysis time. Increase centrifugation speed. Reduce amount of starting material.
Low yield or no DNA	
Sample stored incorrectly	Sample should be stored at 4°C or – 20°C.
Insufficient homogenization of stool sample in Stool DNA Stabilizer	Repeat the DNA purification procedure with a new sample. Be sure to mix the sample in Stool DNA Stabilizer until the sample is thoroughly homogenized. Use Zirconia Beads II 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 sufficiently by pipetting up and down with Binding Buffer A before transferring the sample to the RTA Spin Filter membrane.
No Isopropanol added to Binding Buffer A No alcohol added to the Wash Buffer I and II	Check if Binding Buffer A, Wash Buffer I and Wash Buffer II were diluted with the correct volume of Isopropanol or 96-100% ethanol. Repeat the purification procedure with a new sample.
DNA not eluted efficiently	To increase elution efficiency, pipet the preheated Elution Buffer onto the center of the RTA Spin Filter and incubate the column for 5 minutes at room temperature before centrifugation. Do the elution step twice. Take higher volume of Elution Buffer.
A_{260}/A_{280} 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 Stool DNA Stabilizer	Repeat the procedure with a new sample. Be sure to mix the sample and Stool DNA Stabilizer 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 a new preparation.
DNA does not perform well in downstream applications	
Too much DNA used in downstream reaction	The PSP® Spin Stool DNA Basic Kit 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 or dilute the sample used in the downstream reaction.
Inefficient lysis of bacteria	The amount of target DNA in the eluate may be low if the target organisms 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 PSP® Spin Stool DNA Basic Kit protocol 2, page 17.).
Inhibitory substances in preparation	See section "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 Stool DNA Stabilizer 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 15). *See also protocol: Post Purification, page 28.*
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 Stool DNA Stabilizer 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 15).
Reduced sensitivity of amplification reaction	Determine the maximum volume of eluate for your amplification reaction. Reduce or increase the volume of eluate added to the reaction. Optimize your amplification reaction e.g. by changing template volume.
Precipitation after addition of Binding Buffer A	In most cases, this effect comes from big amounts of DNA in the sample. Do not remove this precipitate and follow strictly the protocol.
General handling	
Lysate not completely passed through silica membrane	Centrifuge for 1 minute at full speed or until all the lysate has passed through the membrane.

Appendix

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 that it can be used well in various downstream applications. Damaged DNA could perform poorly in applications such as genomic Southern blotting, long-template PCR, and construction of cosmid libraries.

Handling fresh and stored material before the extraction of DNA

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

Storage of DNA

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

Drying, dissolving and pipetting DNA

Avoid overdrying genomic DNA after ethanol precipitation. It is better to let it air dry than to use a vacuum, although vacuum drying can be used with caution. Plasmid DNA and other small circular DNAs can be vacuum-dried.

To help dissolve the DNA, carefully invert the tubes several times after adding buffer and tap the tube gently on the side. Alternatively let the DNA stand in buffer overnight at +2 to +8°C. Minimize vortexing of genomic DNA since this can cause shearing.

Avoid vigorous pipetting. Pipetting genomic DNA through narrow 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. Regular pipette tips pose no problem for plasmid DNA and other small fragments.

Determination of concentration, yield, and purity of DNA

Determination of concentration, yield, and purity

DNA yields are determined from the concentration of DNA in the eluate, measured by absorbance at 260 nm. Purity is determined by calculating the ratio of absorbance at 260 nm to absorbance at 280 nm. Pure DNA has an A260/A280 ratio of 1.7–2.1. Absorbance readings at 260 nm should be between 0.1 and 1.0. Sample dilution should be adjusted accordingly. Use elution buffer or water (as appropriate) to dilute samples and to calibrate the spectrophotometer. Measure the absorbance at 260 and 280 nm, or scan absorbance from 220–320 nm (a scan will show if there are other factors affecting absorbance at 260 nm).

Ordering information

Product	Package size	Catalogue No.
PSP® Spin Stool DNA Basic Kit	50 purifications	1038120200
PSP® Spin Stool DNA Basic Kit	250 purifications	1038120300

Product	Package size	Catalogue No.
Stool DNA Stabilizer*)	180 ml	1038111100
Stool Collection Tubes with DNA Stabilizer	50 tubes	1038111200
Stool Collection Tubes with DNA Stabilizer	250 tubes	1038111300

) For 50 reactions one bottle of Stool DNA Stabilizer is suitable, whereas for 250 reactions two bottles are needed.

Related products

InviMag® Stool DNA Kit/ KF96	5 x 96 purifications	7438300200

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

Supplemental not validated protocols, not for diagnostic use

Supplemental protocol for post purification of DNA containing inhibitors

Please read the instructions carefully and carry out preparatory arrangements in advance.

***Important Note*:** *Stool samples are very heterologous, depending on the nutrition of the source organism and the origin of the stool. 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 use water.

2. Sephadex G50 Slurry

Prepare a slurry of Sephadex G50 by adding water to Sephadex G50 powder and soaking until the slurry is reaching its final extension. Depending on the amount you are preparing, it should not take more than 30 minutes.

3. Adsorption of inhibitors

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

4. Removal of slurry

Centrifuge the mixture at 11.000 x g (11.000 rpm) for 1 min. Take the supernatant and transfer it to a new reaction tube, it contains the purified DNA.

This purification may be repeated once, but note that each cleaning step will result in a loss of about 25% of the yield.

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