Instructions for use
PSP® Spin Stool DNA Basic Kit

INVITEK diagnostics

Language: EN

CE IVD

REF 1038120200
1038120300

Σ 50 preparations
250 preparations

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Important notes

Thank you for purchasing the **PSP® Spin Stool DNA Basic Kit** from Invitek Diagnostics.

In combination with the **Stool DNA Stabilizer** or the **Stool Collection Tubes with DNA Stabilizer,** the product serves the purpose of manual isolation of DNA from microorganisms and host from stool samples using Spin Column technology.

WARNING! Improper handling and use for other than the intended purpose can cause danger and damage. Therefore, we ask you to read through these instructions for use and follow them carefully. Always keep them handy. To avoid personal injury, also observe the safety instructions.

All versions of the instructions for use can be found on our website for download or can be requested from us: www.invitek.com

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The kit is in compliance with REGULATION (EU) 2017/746 on *in vitro* diagnostic medical devices. But it is not for *in vitro* diagnostic use in countries where the REGULATION (EU) 2017/746 on *in vitro* diagnostic medical devices is not recognized.

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1. Safety instructions

Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- When and while working with chemicals, always wear protective clothing, disposable gloves and safety glasses.
- Always change pipette tips between liquid transfers. To avoid cross-contamination, we recommend the use of aerosol-barrier pipette tips.
- Do not reuse any consumables.
- Discard gloves if they become contaminated.
- Do not combine components of different kits unless the lot numbers are identical.
- Avoid microbial contamination of the kit reagents.
- To minimize the risk of infections from potentially infectious material, we recommend working under laminar airflow until the samples are lysed.

Before handling chemicals read and understand all applicable Safety data Sheets (MSDS). These are available online at www.invitek.com.

Dispose of kit residues and waste fluids in accordance with your country's regulations, again refer to the MSDS. Invitek Molecular has not tested the liquid waste generated by the kit 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 must be handled and disposed of according to local safety regulations.

European Community risk and safety phrases for the components of the **PSP® Spin Stool DNA Basic Kit** to which they apply are listed below as follows:

Stool DNA Stabilizer

Warning

Hazard statements
H319 - Causes serious eye irritation.
H412 - Harmful to aquatic life with long lasting effects.
Precautionary statements
P264 - Wash hands, forearms and face thoroughly after handling.
P273 - Avoid release to the environment.
P280 - Wear protective gloves/protective clothing/eye protection/face protection/hearing 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.
P337+P313 - If eye irritation persists: Get medical advice/attention.
P501 - Dispose of contents/container to hazardous or special waste collection point, in accordance with local, regional, national and/or international regulation.

Proteinase K

Danger

Hazard statements
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.
Precautionary statements
P261 - Avoid breathing dust/fume/gas/mist/vapours/spray.
P284 - Wear respiratory protection.
P302+P352 - IF ON SKIN: Wash with plenty of water.
P304+P340 - IF INHALED: Remove person to fresh air and keep comfortable for breathing.
P305+P351+P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P501 - Dispose of contents/container to hazardous or special waste collection point, in accordance with local, regional, national and/or international regulation.

Wash Buffer I

Danger

Contains: guanidinium thiocyanate
Hazard statements
H302+H332 - Harmful if swallowed or if inhaled.
H314 - Causes severe skin burns and eye damage.
H412 - Harmful to aquatic life with long lasting effects
Precautionary statements
P260 - Do not breathe dust/fume/gas/mist/vapours/spray.
P271 - Use only outdoors or in a well-ventilated area.
P273 - Avoid release to the environment.
P301+P312 - IF SWALLOWED: Call a POISON CENTRE or doctor if you feel unwell.
P301+P330+P331 - IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.
P303+P361+P353 - IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water.
P501 - Dispose of contents/container to hazardous or special waste collection point, in in accordance with local, regional, national and/or international regulation

Emergency medical information can be obtained 24 hours a day from infotrac, www.infotrac.net:

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

2. Product information

2.1 Kit contents

The **PSP® Spin Stool DNA Basic Kit** does not contain a buffer for sample lysis. For sample lysis it is necessary to combine the **PSP® Spin Stool DNA Basic Kit** with the **Stool DNA Stabilizer** or the **Stool Collection Tubes with DNA Stabilizer** (refer to chapter 2.2 or 4.5 for Ordering information.). The **Stool DNA Stabilizer** has the function of a lysis buffer.

The **Stool DNA Stabilizer** (180 ml bottle) is for direct isolation of fresh or frozen stool samples.

The **Stool Collection Tubes with DNA Stabilizer** are suitable for complete sample management and allow sample collection, transport, stabilization, and storage for up to three months at room temperature (RT).

	50 purifications	**250 purifications**
Catalog No.	1038120200	1038120300
InviAdsorb	50 tubes	5 x 50 tubes
Zirconia Beads II	2 vials	8 vials
Proteinase K	1 vial for 1.5 ml working solution	5 vials for 5 x 1.5 ml working solution
Binding Buffer A	9 ml/bottle (final volume 30 ml)	36 ml/bottle (final volume 120 ml)
Wash Buffer I	30 ml/bottle (final volume 60 ml)	80 ml/bottle (final volume 160 ml)
Wash Buffer II	18 ml/bottle (final volume 60 ml)	2 x 45 ml/bottle (final volume 2 x 150 ml)
Elution Buffer	15 ml/bottle	60 ml/bottle
2.0 ml Safe-Lock-Tubes	2 x 50 tubes	2 x 250 tubes
RTA Spin Filter Set	50 sets	5 x 50 sets
RTA Receiver Tubes	2 x 50 tubes	10 x 50 tubes
1.5 ml Receiver Tubes	2 x 50 tubes	10 x 50 tubes
Short Protocol	1 leaflet	1 leaflet

2.2 Reagents and equipment to be supplied by user

Lab equipment:

- Microcentrifuge (*all protocols were validated with a Centrifuge 5415 D Eppendorf)*
- Optional: centrifuge for 15 or 50 ml
- Thermo shaker (37°C - 95°C)
- Measuring cylinder (250 ml)
- Disposable gloves
- Pipette and pipette tips
- Vortex mixer
- Reaction tubes (1.5 ml, 2.0 ml)

For sample lysis:

Product	Package Size	Catalogue No.
Stool DNA Stabilizer*)	180 ml	1038111100
Stool Collection Tube with DNA Stabilizer	50 tubes	1038111200
Stool Collection Tube 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.**

Liquids and solvents:

- 96 - 100 % ethanol (non-denatured)
- Isopropanol*

*The 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

2.3 Storage, appearance and shelf life

Shelf life: All buffers and kit components should be stored at room temperature and have a shelf life as indicated on the outer kit package label.

After opening, individual components of the kit, as well as components prepared accordingly before first use, have a shelf life of 3 months.

Before each use, make sure that all components are at room temperature. If there are temperature-related precipitates in the solutions, dissolve them by carefully warming (up to 30°C).

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

Wash Buffer I and Wash Buffer II: after adding ethanol, they should be firmly closed and stored at room temperature.

Binding Buffer A: after adding isopropanol, it should be firmly closed and stored at room temperature.

Proteinase K: once dissolved in DNase/RNase free water Proteinase K can be stored at 2 - 8 °C for up to two months. For longer storage keep at –20 °C, freeze-thaw once only.

Stool DNA Stabilizer/Stool Collection Tubes with DNA Stabilizer (to be ordered separately): If there are temperature-related precipitates in the DNA Stabilizer, dissolve them by carefully warming at 30°C in a water bath, shake occasionally during the dissolution process.

2.4 Intended use

The **PSP® Spin Stool DNA Basic Kit** is a Spin Column technology based nucleic acid extraction kit, intended for the isolation and purification of bacterial DNA and host DNA from human stool samples.

The **PSP® Spin Stool DNA Basic Kit** is intended to be used with fresh, frozen or stabilized human stool samples. For fresh or frozen stool samples the product needs to be combined with **Stool DNA Stabilizer**. For comprehensive sample management (sampling, transport) and stabilization at room temperature, the product needs to be combined with **Stool Collection Tubes with DNA Stabilizer**. Adding the Stool DNA Stabilizer to the **PSP® Spin Stool DNA Basic Kit** is essential for sample lysis. **Stool DNA Stabilizer** and **Stool Collection Tubes with DNA Stabilizer** must be purchased separately.

The product is intended for use by professionals only, such as laboratory technicians, physicians and biologists trained in molecular biological techniques and *in vitro* diagnostic procedures.

2.5 Product information and specifications

Starting material	Yield	Quality	Time
Fresh or frozen stool samples: max. 200 mg Stabilized stool samples (Stool Collection Tubes with DNA Stabilizer): 1.4 ml	up to 50 µg, depending on sample (storage and source)	A_{260} : A_{280} 1.7 – 2.1	approx. 45 min (incl. lysis)

Yield and quality of purified DNA from feces is depending on bacteria content, sample source, transport, storage, and age. The donor's state of health can also affect yield and quality, especially in the case of certain illnesses and medication, the quality of the purified nucleic acids can be reduced.

Downstream Applications:
Yield and quality of isolated nucleic acids are in general suitable for plenty of molecular-diagnostic applications such as PCR techniques, microbiome analysis (NGS) and hybridization methods. Downstream applications should be performed according to the respective manufacturers' specifications.

2.6 Principle and procedure

1. Lyse samples
Stool samples are lysed in Stool DNA Stabilizer under denaturing conditions and different temperature levels specific for the target nucleic acid. Human cells for host DNA isolation are lysed at RT, while bacterial cells must be incubated at higher temperatures. For lysis of bacterial cells Zirconia beads are added to increase lysis efficiency.

2. Removal of PCR inhibitors and protein digestion
After lysis, DNA degrading substances and PCR inhibitors present in the feces are adsorbed to the InviAdsorb matrix. InviAdsorb is in pre-filled Safe-Lock tubes into which the lysate must be placed. The bound contaminants and cell debris are pelleted by centrifugation. The supernatant contains the pre-purified DNA.
Proteinase K is added to the supernatant to digest and degrade proteins at elevated temperature.

3. Bind nucleic acids
By adding Binding Buffer A to the lysate, optimal binding conditions are adjusted. Each lysate is then applied to an RTA Spin Filter and nucleic acids are adsorbed to the membrane.

4. Wash to remove residual contaminations
Contaminants are efficiently washed away using Wash Buffer I and Wash Buffer II, while nucleic acids remain bound to the membrane.

5. Elute DNA
Nucleic acids are eluted from the RTA Spin Filter using 100 - 200 µl Elution Buffer.

3. Nucleic acid extraction with the PSP® Spin Stool DNA Basic Kit

3.1 Before starting a protocol

When using the kit for the first time make sure all buffers and reagents are prepared as indicated:

Buffer preparations prior first use: 50 preparations
Binding Buffer A: Fill 21 ml **99.7% isopropanol** (molecular biology grade) into the bottle, mix by shaking vigorously for 1 minute. Mix by inverting several times just before use. Always keep the bottle firmly closed. **Proteinase K:** Resuspend lyophilized Proteinase K by addition of 1.5 ml **DNase/RNase free water** to the vial, mix thoroughly until completely dissolving. **Wash Buffer I:** Add 30 ml of **96 -100% ethanol** to the bottle**.** Mix thoroughly, always keep the bottle firmly closed. **Wash Buffer II:** Add 42 ml of **96 -100% ethanol** to the bottle. Mix thoroughly, always keep the bottle firmly closed.
Buffer preparations prior first use: 250 preparations
Binding Buffer A: Fill 84 ml **99.7% isopropanol** (molecular biology grade) into the bottle, mix by shaking vigorously for 1 minute. Mix by inverting several times just before use. Always keep the bottle firmly closed. **Proteinase K:** Resuspend lyophilized Proteinase K by addition of 1.5 ml **DNase/RNase free water** to each vial, mix thoroughly until completely dissolving. **Wash Buffer I:** Add 80 ml of **96 -100% ethanol** to the bottle**.** Mix thoroughly, always keep the bottle firmly closed. **Wash Buffer II:** Add 105 ml of **96 -100% ethanol** to the bottle. Mix thoroughly, always keep the bottle firmly closed.

- Adjust the thermo shaker to 70°C.
- Adjust thermo shaker/heating blocks to 70°C and 95°C
- Warm up the needed amount of **Elution Buffer** to 70°C
  (100 - 200 µl **Elution Buffer** are needed per sample).
- Determine the number of required reactions including controls and label the needed amount of RTA Spin Filters (lid) and the needed amount of 1.5 ml Receiver Tubes (per sample: 1 Receiver Tube is needed).

3.2 Sampling and storage of starting material

For reproducible and high yields, the correct sample storage is essential. Yields may vary depending on factors such as nutritional status of the donor, sample age, sample type, transport and storage.

Repeated freeze-thaw cycles of samples should be avoided to prevent nucleic acid degradation. In general, best results are obtained using fresh samples. It is recommended to consider technical guidance such as e.g., CEN/TS and ISO standards on the pre-examination process for molecular diagnostics under IVDR as highlighted in G. Dagher, et al. (https://doi.org/10.1016/j.nbt.2019.05.002).

Fresh stool samples: Samples contain DNases which can quickly cause DNA degradation. Fresh stool samples can be stored for 1-2 hours at RT, Stool DNA Stabilizer should be added to the sample as soon as possible. Otherwise, the samples should be stored frozen at – 80°C.

Stabilized stool samples: Stool samples can be stabilized using the Stool Collection Tubes with DNA Stabilizer from Invitek Diagnostics (refer to Ordering information) which allows sampling, storage and transport of the sample. For sampling, 1 g of stool is collected with the spoon which is integrated in the lid of the Collection Tube. After sampling the Collection Tube must be firmly closed. The sample should be mixed by thoroughly shaking or vortexing to homogenize the sample with the Stool DNA Stabilizer in the Tube. Samples can be stored in Stool Collection Tubes with DNA Stabilizer for up to 3 months at RT. Storage time below 3 months has no influence on the quality or the amount of DNA. For long term storage samples can be frozen at -20°C or -80°C. Aliquotation of samples prior freezing is recommended.

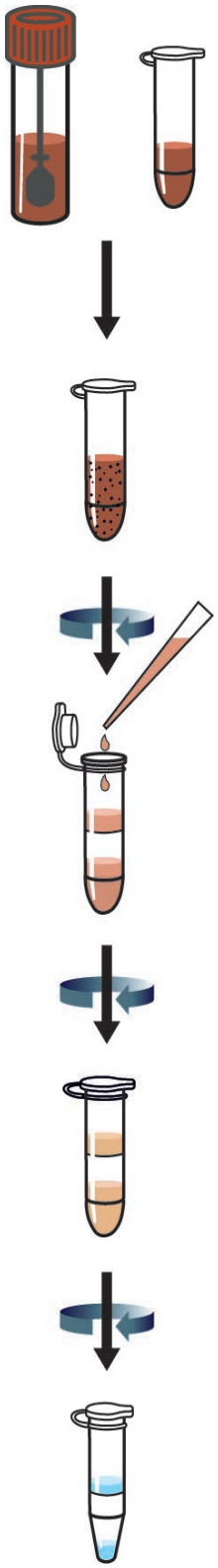
3.3 Preparation of starting materials

Fresh stool samples, liquid: If the sample is liquid, pipet 200 µl into a 2.0 ml Safe-Lock-Tube. Cut off the end of the pipet tip to make pipetting easier. Proceed with adding Stool DNA Stabilizer as described in the extraction protocol.

Frozen stool samples: use a scalpel or spatula to scrape bits of stool into a 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.

Samples in Stool Stabilizer may be thawed directly by careful warming, please avoid multiple freeze thaw cycles, which may lead to DNA shearing, by aliquoting the respective samples.

3.4 Short protocol PSP® Spin Stool DNA Basic Kit



Lyse samples

1. **Fresh/frozen samples:** Weigh 200 mg of stool sample (fresh or frozen) into a 2.0 ml Safe-Lock-Tube, add 1.2 ml Stool DNA Stabilizer, vortex vigorously for 1 min. **Stabilized samples:** Transfer 1.4 ml sample from the Stool Collection Tube with DNA Stabilizer after storage or directly after sampling into a 2.0 ml Safe-Lock-Tube.

   a) **Isolation of host DNA**
   Incubate 10 min at RT continuously shaking at 900 rpm
   Centrifuge for 1 min at 11.000 x g

   b) isolation of bacterial DNA
   Incubate 10 min at 95°C continuously shaking at 900 rpm.
   Add 5 **Zirconia Beads II** to the homogenate and vortex for 2 min
   Centrifuge for 1 min at 11.000 x g

   c) isolation of DNA from difficult to lyse bacteria
   Incubate 10 min at 95°C continuously shaking at 900 rpm
   Incubate 3 min on ice
   Add 5 **Zirconia Beads II** and incubate 3 min at 95°C.Vortex the sample 2 min, centrifuge for 1 min at 11.000 x g

Removal of PCR inhibitors and protein digestion

2. Transfer the supernatant into an **InviAdsorb-Tube,** vortex vigorously for 15 sec
   Incubate for 1 min at RT.Centrifuge for 3 min at full speed.
3. Transfer the supernatant completely into a new 1.5 ml Receiver Tube, discard the pellet with the InviAdsorb. Centrifuge for 3 min at full speed.
4. Transfer 25 µl **Proteinase K** into a new 2.0 ml Safe-Lock-Tube, pipette 400 µl or **800 µl for stabilized samples** of the supernatant from step 3 into the Safe-Lock-Tube containing **Proteinase K**, mix shortly by vortexing. Incubate for 10 min at 70°C continuously shaking at 900 rpm.

Bind genomic DNA

5. Add 200 µl **Binding Buffer A or 400 µl Binding Buffer A for stabilized samples** to the lysate, mix shortly by vortexing or by pipetting up and down several times
   Take an RTA Spin Filter Set. Transfer the mixture completely **(in two steps for stabilized samples)** to the RTA Spin Filter.
   Close the RTA Spin Filter and incubate for 1 min at RT.
   Centrifuge for 2 min at 11.000 x g. Discard the filtrate and place the RTA Spin Filter in a new 2.0 ml RTA Receiver Tube.

Wash to remove residual contaminations

6. Add 500 µl **Wash Buffer I,** centrifuge for 1 min at 11.000 x g. Discard the filtrate and place the RTA Spin Filter in a new 2.0 ml RTA Receiver Tube
7. Add 700 µl **Wash Buffer II,** centrifuge for 1 min at 11.000 x g
   Discard the filtrate and place the RTA Spin Filter **back to** the 2.0 ml RTA Receiver Tube
8. Centrifuge for 4 min at maximum speed to eliminate the ethanol completely
   Discard the RTA Receiver Tube

Elute DNA

9. Place the RTA Spin Filter in a 1.5 ml Receiver Tube. Add 100-200 µl of the preheated (70°C) **Elution Buffer**
   Incubate 1 min at RT
   Centrifuge for 1 min at 11.000 x g to elute DNA. Discard the RTA Spin Filter

3.5 Protocol 1: Isolation of DNA from fresh or frozen stool samples

1. 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 sample. Vortex vigorously for 1 min. Also for smaller sample volumes use the same amount of **Stool DNA Stabilizer**.

   a) Isolation of host DNA
   Incubate 10 min at RT continuously shaking at 900 rpm.
   Centrifuge for 1 min at 11.000 x g to pellet solid stool particles.

   b) isolation of bacterial DNA
   Incubate 10 min at 95°C continuously shaking at 900 rpm.
   Add 5 **Zirconia Beads II** to the homogenate and vortex for 2 min.
   Centrifuge for 1 min at 11.000 x g to pellet solid stool particles and Beads.

   c) isolation of DNA from difficult to lyse bacteria
   Incubate 10 min at 95°C continuously shaking at 900 rpm.
   Incubate 3 min on ice.
   Add 5 **Zirconia Beads II** to the homogenate and incubate 3 min at 95°C.
   Vortex the sample 2 min, centrifuge for 1 min at 11.000 x g to pellet solid stool particles and Beads.

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

3. Transfer the supernatant completely into a new 1.5 ml Receiver Tube, discard the pellet with the InviAdsorb.
   Centrifuge for 3 min at full speed.

4. Transfer 25 µl **Proteinase K** into a new 2.0 ml Safe-Lock-Tube and pipette 400 µl of the supernatant from step 3 into the Safe-Lock-Tube containing **Proteinase K**, mix shortly by vortexing.
   Incubate for 10 min at 70°C continuously shaking at 900 rpm.

5. Add 200 µl **Binding Buffer A** to the lysate and mix shortly by vortexing or by pipetting up and down several times.
   Take a RTA Spin Filter Set. Transfer the mixture completely into the RTA Spin Filter.
   Close the RTA Spin Filter and incubate for 1 min at RT.
   Centrifuge for 2 min at 11.000 x g.
   Discard the filtrate and place the RTA Spin Filter in a new 2.0 ml RTA Receiver Tube.

6. Add 500 µl **Wash Buffer I** and centrifuge for 1 min at 11.000 x g.
   Discard the filtrate and place the RTA Spin Filter in a new 2.0 ml RTA Receiver Tube.

7. Add 700 µl **Wash Buffer II** and centrifuge for 1 min at 11.000 x g.
   Discard the filtrate and place the RTA Spin Filter **back to** the 2.0 ml RTA Receiver Tube.

8. Centrifuge for 4 min at maximum speed to eliminate the ethanol completely.
   Discard the RTA Receiver Tube.

9. Place the RTA Spin Filter in a 1.5 ml Receiver Tube. Add 100-200 µl of the preheated (70°C) **Elution Buffer**.
   Incubate 1 min at RT.
   Centrifuge for 1 min at 11.000 x g to elute DNA. Discard the RTA Spin Filter.

Note: *DNA can also be eluted in a lower volume of Elution Buffer (depending on the expected yield). The minimum volume for elution is 50 µl, note that using a low elution volume can reduce the maximum yield. If a large amount of DNA is expected, the volume of elution can also be increased.*

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

3.6 Protocol 2: Isolation of DNA from stabilized stool samples

1. Collect the sample with the **Stool Collection Tube with DNA Stabilizer.**
   Transfer 1.4 ml of the well homogenized stool sample from the Collection Tube after storage or directly after sampling into a 2.0 ml Safe-Lock-Tube.

   a) Isolation of host DNA
   Centrifuge for 1 min at 11.000 x g to pellet solid stool particles.

   b) isolation of bacterial DNA
   Incubate 10 min at 95°C continuously shaking at 900 rpm.
   Add 5 **Zirconia Beads II** to the homogenate and vortex for 2 min.
   Centrifuge for 1 min at 11.000 x g to pellet solid stool particles and Beads.

   c) isolation of DNA from difficult to lyse bacteria
   Incubate 10 min at 95°C continuously shaking at 900 rpm.
   Incubate 3 min on ice.
   Add 5 **Zirconia Beads II** to the homogenate
   Incubate 3 min at 95°C.
   Vortex the sample 2 min, centrifuge for 1 min at 11.000 x g to pellet solid stool particles and Beads.
2. Transfer the supernatant into an **InviAdsorb-Tube** and vortex vigorously for 15 sec.
   Incubate for 1 min at RT.
   Centrifuge for 3 min at full speed.
3. Transfer the supernatant completely into a new 1.5 ml Receiver Tube, discard the pellet with the InviAdsorb.
   Centrifuge for 3 min at full speed.
4. Transfer 25 µl **Proteinase K** into a new 2.0 ml Safe-Lock-Tube and pipette 800 µl of the supernatant from step 3 into the Safe-Lock-Tube containing **Proteinase K**, mix shortly by vortexing.
   Incubate for 10 min at 70°C continuously shaking at 900 rpm.
5. Add 400 µl **Binding Buffer A** to the lysate and mix shortly by vortexing or by pipetting up and down several times.
   Take a RTA Spin Filter Set. Transfer the mixture completely, **in two steps,** into the RTA Spin Filter.
   Close the RTA Spin Filter and incubate for 1 min at RT.
   Centrifuge for 2 min at 11.000 x g.
   Discard the filtrate and place the RTA Spin Filter in a new 2.0 ml RTA Receiver Tube.
6. Add 500 µl **Wash Buffer I** and centrifuge for 1 min at 11.000 x g.
   Discard the filtrate and place the RTA Spin Filter in a new 2.0 ml RTA Receiver Tube.
7. Add 700 µl **Wash Buffer II** and centrifuge for 1 min at 11.000 x g.
   Discard the filtrate and place the RTA Spin Filter **back to** the 2.0 ml RTA Receiver Tube.

8. Centrifuge for 4 min at maximum speed to eliminate the ethanol completely. Discard the RTA Receiver Tube.
9. Place the RTA Spin Filter in a 1.5 ml Receiver Tube. Add 100-200 µl of the preheated (70°C) **Elution Buffer**.
   Incubate 1 min at RT.
   Centrifuge for 1 min at 11.000 x g to elute DNA. Discard the RTA Spin Filter.

Note: *DNA can also be eluted in a lower volume of Elution Buffer (depending on the expected yield). The minimum volume for elution is 50 µl, note that using a low elution volume can reduce the maximum yield. If a large amount of DNA is expected, the volume of elution can also be increased.*

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

4. Appendix

4.1 Troubleshooting

Problem	Possible cause	Recommendation
Clogged RTA Spin Filter	Insufficient cell lysis and/or too much starting material	Increase lysis time Increase centrifugation speed Reduce amount of starting material
RNA contamination	The RTA Spin Filter can purify low amounts of RNA	Add 20 µl RNase A (10 mg/ml) and incubate for 10 minutes before adding Binding Buffer A.
Low amount of nucleic acids	Insufficient cell lysis	Increase lysis time Reduce amount of starting material to avoid column overload Prolong incubation time at 95°C, use Zirconia Beads
	Incomplete elution	Increase incubation time with preheated **Elution Buffer** to 5 min Elute twice with 100 µl **Elution Buffer** Use higher volume of **Elution Buffer**
	Low nucleic acid-concentration in the sample	Elute the DNA with a lower volume of **Elution Buffer**, do not use volumes below 50 µl
	Incorrect storage of starting material	Ensure that starting material is appropriately stored. Avoid repeated thaw-freeze cycles of the sample material.
	Buffers were incorrectly prepared	Ensure, that the correct amount of ethanol/isopropanol is added to the Buffers and that all solutions are stored firmly closed.
	Insufficient homogenization of sample and 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 mixing of the sample with Binding Buffer A	Mix sample properly by pipetting before transferring the sample to the RTA Spin Filter membrane.
Degraded nucleic acids/ low A_{260}/A_{280} ratio	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.
	Decreased Proteinase activity	Repeat the DNA purification procedure with a new sample and with Proteinase K. For difficult cases use double volume Proteinase K.
	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.
Nucleic acids do not perform well in downstream-applications (e.g. real-time PCR or NGS)	Too much DNA used in downstream reaction	The extracted DNA can come from many different organisms present in the original stool sample (e.g. human, animal, plant, bacteria). Reduce the amount of eluate or dilute the sample used in the downstream reaction.
	Insufficient cell lysis	See above

4.2 Warranty

Invitek Molecular guarantees the correct function of the kit for applications described in this manual and in accordance with the intended use. In accordance with Invitek Molecular' EN ISO 13485 certified Quality Management System the performance of all kit components has been tested to ensure product quality.
Any problems, incidents or defects shall be reported to Invitek Molecular immediately upon detection. Immediately upon receipt, inspect the product to ensure that it is complete and intact. In the event of any discrepancies, you must inform Invitek Molecular immediately in writing. Modifications of the kit and protocols and use that deviate from the intended purpose are not covered by any warranty.

Invitek Molecular reserves the right to change, alter, or modify any product to enhance its performance and design at any time.
Invitek Molecular warrants products as set forth in the General Terms and Conditions available at www.invitek.com.
If you have any questions, please contact techsupport@invitek.com.

4.3 Symbols used on product and labeling

 Manufacturer

 Lot number

 Unique identifier of a medical device

 Catalogue number

 Expiry date

 Consult operating instructions

 Temperature limitation

 Do not reuse

 Amount of sample preparations

 in vitro diagnostic medical device

4.4 Further documents and supplementary information

Visit www.invitek.com for further information on:

- FAQs and troubleshooting tips
- Manuals in different languages
- Safety data Sheets (MSDS)
- Web support
- Product videos

If, despite careful study of the operating instructions and further information, you still require assistance, please contact us at techsupport@invitek.com or the dealer responsible for you.

4.5 Ordering information

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

For sample lysis:

Product	Package Size	Catalogue No.
Stool DNA Stabilizer*)	180 ml	1038111100
Stool Collection Tube with DNA Stabilizer	50 tubes	1038111200
Stool Collection Tube 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.**

Revision history

Revision	Date	Description
EN-v1-2022	2022-06-15	New document
EN-v2-2023	2023-04-14	Update contact details and corporate design to reflect the company's rebranding.
EN-v3-2023	2023-12-11	Correction of DNA quality specifications.
EN-v4-2024	2024-01-24	Update hazard and precautionary statements

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2024-01-24 EN-v4-2024