

Instructions for use
InviMag® Stool DNA Kit/ KF96

INVITEK
diagnostics



InviMag®

Language: EN

RUO

REF 7438300200

Σ 5 x 96 preparations

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Portugal

Important notes

Thank you for purchasing the **InviMag® Stool DNA Kit/ KF96** from Invitek Diagnostics.

The product serves the purpose of semi-automated isolation of genomic DNA from fresh, frozen or stabilized stool samples, in a 96-well format using magnetic beads 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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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 Diagnostics 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 **InviMag® Stool DNA Kit/ KF96** to which they apply are listed below as follows:

Proteinase K



Danger

H315-H319-H334-H335
P280-P305+P351+P338

Lysis Buffer P



Warning

H319-H412
P280-P305+P351+P338-P273

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.

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, www.infotrac.net:

outside of USA: 1 – 352 – 323 – 3500

in USA: 1 – 800 – 535 – 5053

2. Product information

2.1 Kit contents

	InviMag® Stool DNA Kit/KF96 5 x 96 preparations
Catalogue No.	7438300200
Lysis Buffer P	3 x 210 ml/bottle
Binding Buffer A	2 x 24 ml/bottle (final volume 2 x 80 ml)
Zirconia Beads II	14 vials
SNAP Solution	10.5 ml/bottle
Proteinase K	10 tubes (final volume 1.5 ml working solution)
Wash Buffer I	3 x 80 ml/bottle (final volume 3 x 160 ml)
Wash Buffer II	5 x 60 ml/bottle (final volume 5 x 200 ml)
Elution Buffer	120 ml/bottle
InviAdsorb	10 x 50 tubes
1.5 ml Receiver Tube	10 x 50 tubes
2.0 ml Deep Well Plate	4 x 5 plates
KF96 Tip Comb for DW magnets	5 tip combs
200 µl Elution Plate	5 x 2 plates
Short Protocol	1 leaflet

2.2 Reagents and equipment to be supplied by user

Lab equipment:

- KingFisher™ Flex with 96 Deep-Well Head and consumables:
KingFisher 96, Magnetic Particle Processor, 100-240V, 50/60Hz with Deep well Head (available at ThermoFisher Scientific)
- Microcentrifuge
- Measuring cylinder (250 ml)
- Disposable gloves
- Pipette and pipette tips (filter tips are recommended)
- Reagent reservoirs for working with multichannel pipettes
- Vortex mixer
- Thermo mixer (95°C)

For sample collection and stabilization:

Product	Package Size	Catalogue No.
Stool Collection Tube with DNA Stabilizer	50 tubes	103811 1200
Stool Collection Tube with DNA Stabilizer	250 tubes	103811 1300

Liquids and solvents:

- DNase/RNase free water
- 96 – 100 % ethanol (non-denatured)
- Isopropanol

2.3 Storage, appearance and shelf life

Shelf life: All buffers and kit components should be stored at room temperature, unless stated otherwise, 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 Buffers: 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.

2.4 Intended use

The **InviMag® Stool DNA Kit/ KF96** is for the semi-automated simultaneous isolation and purification of total DNA from the host or microorganisms, in a 96-well format using magnetic beads technology.

The kit is intended to be used with fresh, frozen or stabilized human or animal stool samples. For comprehensive sample management (sampling, transport) and stabilization at room temperature, the product needs to be combined with **Stool Collection Tubes with DNA Stabilizer** (available separately, refer to chapter 4.6 "Ordering information").

The **InviMag® Stool DNA Kit/ KF96** is validated for the use on a KingFisher™ Flex (Thermo Fisher Scientific) instrument. Ensure correct function and configuration of the instrument according to the manufacturer's instructions. Improper use of the instrument may result in lower yields and can potentially harm the instrument.

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: 200 mg	Up to 50 µg, depending on sample (storage and source)	A ₂₆₀ : A ₂₈₀ 1.7 – 2.0	approx. 50 min. (incl. lysis)
Stabilized stool samples (Stool Collection Tubes with DNA Stabilizer): 1.4 ml			

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

The KingFisher™ Flex instrument uses magnetic rods to transport paramagnetic beads with bound nucleic acids through the different extraction phases: lysis, binding, washing and elution. The semi-automated purification process provides a reproducible method for obtaining highly pure nucleic acids.

1. Lyse samples

Stool samples are lysed in Lysis Buffer P or Stool DNA Stabilizer, depending on the mode of sampling. Cells for host DNA isolation are lysed at room temperature, while bacterial cells must be incubated at 95°C. 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 supernatant, optimal binding conditions are adjusted. Additionally SNAP Solution, containing silica coated magnetic beads, is added. Nucleic acids bind specifically to the magnetic beads.

4. Wash to remove residual contaminations

Contaminants are efficiently washed away in three washing steps using Wash Buffer I and Wash Buffer II, while nucleic acids remain bound to magnetic beads.

5. Elute DNA

Nucleic acids are released from magnetic beads and are eluted in 150 µl Elution Buffer.

3. Nucleic acid extraction with the InviMag® Stool DNA Kit /KF96

3.1 Before starting a protocol

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

Buffer preparations prior first use:
Binding Buffer A: Add 56 ml 99.7% isopropanol (molecular biology grade) into the bottle, mix by shaking for 1 min. Always keep the bottle firmly closed.
Wash Buffer I: Add 80 ml of 96 -100% ethanol to each bottle. Mix thoroughly, always keep the bottle firmly closed.
Wash Buffer II: Add 140 ml of 96 -100% ethanol to each bottle. Mix thoroughly, always keep the bottle firmly closed.
Proteinase K: Resuspend each tube in 1.5 ml DNase/RNase free water . Mix thoroughly until completely dissolved.

- Adjust thermo shaker/heating blocks to 95°C
- **Binding Buffer A:** Shortly before use mix by inverting several times
- **SNAP Solution:** Shortly before use mix by vortexing

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 health 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.

Fresh stool samples: Samples contain DNases which can quickly cause DNA degradation. Fresh stool samples can be stored for 1-2 hours at RT, Lysis Buffer P or 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 material

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.

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 Lysis Buffer P is added, otherwise the DNA in the sample may degrade.

3.3.1 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 **Lysis Buffer P** to each sample. Vortex vigorously for 1 min. Also for smaller sample volumes use the same amount of **Lysis Buffer P**.

a) isolation of host DNA

Incubate 10 min at RT continuously shaking at 900 rpm.

Centrifuge for 1 min at 13.000 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 at RT.

Centrifuge for 1 min at 13.000 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 13.000 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 25 µl **Proteinase K** into the cavities of the **Binding Plate** and add 400 µl of the supernatant from step 2.
4. Continue as described in chapter 3.5 "Preparing and loading the KingFisher™ Flex".

3.3.2 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 13.000 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 at RT.
Centrifuge for 1 min at 13.000 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 13.000 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 25 µl **Proteinase K** into the cavities of the **Binding Plate** and add 400 µl of the supernatant from step 2.
4. Continue as described in chapter 3.5 “Preparing and loading the KingFisher™ Flex”

3.5 Preparing and loading the KingFisher™ Flex

When operating the KingFisher™ Flex make sure you have read and understood the manufacturer's instructions.

1. Determine the number of needed reactions including controls and prepare all plates needed for the purification procedure as follows. Label the short side of each plate accordingly.

Plate setup for KingFisher™ Flex:	
Binding Plate	Add 25 µl Proteinase K and 400 µl pre-treated sample. Refer to chapter 3.3 "Preparation of starting material" for sample specific pre-treatment.
Washing plate_1	Add 800 µl Wash Buffer I into the cavities of a Deep Well Plate
Washing plate_2	Add 800 µl Wash Buffer II into the cavities of a Deep Well Plate
Washing plate_3	Add 800 µl Wash Buffer II into the cavities of a Deep Well Plate
Elution Plate	Add 150 µl Elution Buffer into the cavities of an Elution Plate

2. Binding Plate: Prepare the Binding Plate according to the sample type, as described in "Preparation of starting material".
3. Switch on the KingFisher™ Flex instrument.
4. Tip Plate: Place the KF96 Tip Comb for DW magnets on a tip plate (Tip Plate and Elution Plate are identical).
5. Choose the assay for starting the run (the assay is available for download at the Invitex Diagnostics web page).

InviMag_Stool_DNA_KFflex96_SNAP: protocol for KingFisher™ Flex instrument.

6. Load prepared plates into the position specified in the display of the instrument.
7. "START" the run.
8. After a 20 min. lysis step, the instrument pauses and 300 µl **Binding Buffer A** and 20 µl **SNAP Solution** must be added to each sample containing cavity.

Note: *Invert Binding Buffer A several times before use!*

Mix SNAP Solution before use by vortexing vigorously!

Place the plate back to the instrument (watch out for correct plate orientation) and continue the run by pressing the "START" button. The instrument will now continue the purification process without any further user interaction.

9. After extraction: A transfer of purified nucleic acids to 1,5 ml Receiver Tubes (provided) is recommended.

Keep nucleic acids at -20°C or -80°C until further use.

Note: *The elution volume can be decreased to a minimum of 75 µl which will result in a higher yield. When applying changes to the elution volume, also change the run file accordingly.*

4. Appendix

4.1 Step-by-step protocol of the KingFisher™ Flex

Protocol: InviMag_Stool_DNA_KFflex96_SNAP







Reagent info

Tip Plate		KingFisher 96 KF plate		
Name	Well volume [µl]	Total reagent volume [µl]	Type	
-	-	-	-	
Binding Plate		Microtiter DW 96 plate		
Name	Well volume [µl]	Total reagent volume [µl]	Type	
Prelysed Sample	400	-	Sample	
Proteinase K	25	-	Reagent	
Washing Plate 1		Microtiter DW 96 plate		
Name	Well volume [µl]	Total reagent volume [µl]	Type	
Wash Buffer I	800	-	Reagent	
Washing Plate 2		Microtiter DW 96 plate		
Name	Well volume [µl]	Total reagent volume [µl]	Type	
Wash Buffer II	800	-	Reagent	
Washing Plate 3		Microtiter DW 96 plate		
Name	Well volume [µl]	Total reagent volume [µl]	Type	
Wash Buffer II	800	-	Reagent	
Elution Plate		KingFisher 96 KF plate		
Name	Well volume [µl]	Total reagent volume [µl]	Type	
Elution Buffer	150	-	Reagent	

Dispensed reagents

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

Steps data

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

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

4.2 Troubleshooting

Problem	Possible cause	Recommendation
Low amount of nucleic acids	Insufficient lysis	Increase lysis time in the provided run file. Reduce amount of starting material.
	Insufficient mixing of the sample with Binding Buffer A	Mix sample with Binding Buffer A by mixing up and down before adding the beads to the sample.
	Incomplete elution	Elongate elution time or elevate elution buffer volume in protocol and run file
	Low amount of SNAP Solution	Mix SNAP Solution thoroughly before use.
	Insufficient sample homogenization in Lysis Buffer P or Stool DNA Stabilizer	Make sure the sample is properly homogenized in Lysis Buffer P/Stool DNA Stabilizer. For difficult to homogenize samples use Zirkonia Beads and vortex for homogenization.
	Incorrect storage of starting material, old 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.
DNA does not perform well in downstream-applications (e.g. real-time PCR or NGS)	Ethanol carryover during elution	Increase time of drying step for removal of ethanol in the run file.
	Inhibitory compounds in the eluate	Make to sure to mix the sample well with the InviAdsorb matrix until the sample is thoroughly homogenized. Perform a clean-up of the eluate using Sephadex 50 (Cytiva).
	Too much DNA used in downstream reaction	Reduce the DNA concentration in downstream reactions to avoid inhibition or unspecific PCR products.
Magnetic beads carry-over	Residues of magnetic particles in eluted extraction	Centrifuge eluted nucleic acids at full speed for 1 min and transfer supernatant to a new tube.

4.3 Warranty

Invitek Diagnostics guarantees the correct function of the kit for applications described in this manual and in accordance with the intended use. In accordance with Invitek Diagnostics's EN ISO 13485 and ISO 9001 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 Diagnostics 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 Diagnostics immediately in writing. Modifications of the kit and protocols and use that deviate from the intended purpose are not covered by any warranty.

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

Invitek Diagnostics 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

4.4 Symbols used on product and labeling



Manufacturer



Lot number



Catalogue number



Expiry date



Consult operating instructions



Temperature limitation



Do not reuse



Amount of sample preparations



Research Use Only

4.5 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
- Run-Files for KingFisher™ Flex and KingFisher™ 96

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.

4.6 Ordering information

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

For sample collection and stabilization:

Product	Package Size	Catalogue No.
Stool Collection Tube with DNA Stabilizer	50 tubes	1038111200
Stool Collection Tube with DNA Stabilizer	250 tubes	1038111300

Revision history

Revision	Date	Description
DE 575.01	2025-07-31	New document



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