Instructions for Use
Life Science Kits & Assays

innuPREP Stool DNA Kit

Innuscreen GmbH

Order No.:

845-KS-7010010 10 reactions

845-KS-7010050 50 reactions

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This documentation describes the state at the time of publishing.
It needs not necessarily agree with future versions. Subject to change!

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1 Introduction

1.1 Intended use

The innuPREP Stool DNA Kit has been designed as a tool for isolation of bacterial and host DNA from stool samples. The kit is intended for use by professional users. The kit has been designed to be used for a wide range of different downstream applications like amplification reactions and further analytical procedures.

CONSULT INSTRUCTION FOR USE
This package insert must be read carefully before use. Package insert instructions must be followed accordingly. Reliability of results cannot be guaranteed if there are any deviations from the instructions in this package insert.

1.2 Notes on the use of this manual and the kit

For easy reference and orientation, the manual and labels use the following warning and information symbols as well as the shown methodology:

Symbol	Information
REF	**REF** Catalogue number
Σ N	**Content** Contains sufficient reagents for <N> tests
15 °C 30 °C	**Storage conditions** Store at room temperature or shown conditions respectively
	Consult instructions for use This information must be observed to avoid improper use of the kit and the kit components.
	Expiry date
LOT	**Lot number** The number of the kit charge
	Manufactured by Contact information of manufacturer
	For single use only **Do not use components for a second time**
	Note / Attention Observe the notes marked in this way to ensure correct function of the device and to avoid operating errors for obtaining correct results.

The following systematic approach is introduced in the manual:

- The chapters and figures are numbered consecutively.
- A cross reference is indicated with an arrow (e.g. → "Notes on the use of this manual" p.3).
- Work steps are numbered.

2 Safety precautions

NOTE

Read through this chapter carefully before to guarantee your own safety and a trouble-free operation.

Follow all the safety instructions explained in the manual, as well as all messages and information, which are shown.

All due care and attention should be exercised in handling the materials and reagents contained in the kit. Always wear gloves while handling these reagents and avoid any skin contact! In case of contact, flush eyes or skin with a large amount of water immediately.

FOR SINGLE USE ONLY!

This kit is made for single use only!

ATTENTION!

Don’t eat or drink components of the kit!

The kit shall only be handled by educated personnel in a laboratory environment!

If the buffer bottles are damaged or leaking, wear gloves and protective goggles when discarding the bottles in order to avoid any injuries. This kit is to be used with potential infectious samples. Therefore, all liquid waste must be considered as potentially infectious and must be handled and discarded according to local safety regulation.

Please observe the federal, state and local safety and environmental regulations. Follow the usual precautions for applications using extracted nucleic acids. All materials and reagents used for DNA or RNA isolation should be free of DNases or RNases.

ATTENTION!

Do not add bleach or acidic components to the waste after sample preparation!

NOTE

Emergency medical information in English and German can be obtained 24 hours a day from:

Poison Information Center, Freiburg / Germany

Phone: +49 (0)761 19 240.

For more information on GHS classification and the Safety Data Sheet (SDS) please contact sds.innu@ist-ag.com.

3 Storage conditions

The kit is shipped at ambient temperature.

Upon arrival, store lyophilized and dissolved Proteinase K at 4 °C to 8 °C.

All other components of the innuPREP Stool DNA Kit should be stored dry at room temperature (15 °C to 30 °C). When stored at room temperature, the kit is stable until the expiration date printed on the label on the kit box.

If there are any precipitates within the provided solutions solve these precipitates by careful warming. Before every use make sure that all components have room temperature.

For further information see chapter “Kit components” (→ p.7).

4 Functional testing and technical assistance

The IST Innuscreen GmbH guarantees the correct function of the kit for applications as described in the manual. This product has been produced and tested in an ISO 13485 certified facility.

We reserve the right to change or modify our products to enhance their performance and design. If you have any questions or problems regarding any aspects of the innuPREP Stool DNA Kit or other IST Innuscreen GmbH products, please do not hesitate to contact us. For technical support or further information in Germany please dial +49 30 9489 3380. For other countries please contact your local distributor.

5 Product use and warranty

The kit is not designed for the usage of other starting materials or other amounts of starting materials than those, referred to in the manual (→ “Intended use” p.2) (→ “Product specifications” p.8). Since the performance characteristics of IST Innuscreen GmbH kits have just been validated for the application described above, the user is responsible for the validation of the performance of IST Innuscreen GmbH kits using other protocols than those described below. IST Innuscreen GmbH kits may be used in clinical diagnostic laboratory systems after the laboratory has validated the complete diagnostic system as required by CLIA’ 88 regulations in the U.S. or equivalent regulations required in other countries.

All products sold by the IST Innuscreen GmbH are subjected to extensive quality control procedures and are warranted to perform as described when used correctly. Any problems should be reported immediately.

NOTE

The kit is for research use only!

6 Kit components

6.1 Included kit components

	Σ 10	Σ 50
REF	845-KS-7010010	845-KS-7010050
Lysis Solution SLS	12 ml	60 ml
Binding Solution SBS	5 ml	20 ml
Proteinase K	for 1 x 0.3 ml working solution	for 1 x 1.5 ml working solution
Washing Solution HS (conc.)	5 ml	20 ml
Washing Solution MS (conc.)	3 ml	15 ml
Elution Buffer	2 x 2 ml	15 ml
Prefilter	10	50
Spin Filter	10	50
Receiver Tubes	60	6 x 50
Elution Tubes	10	50
Manual	1	1

6.2 Components not included in the kit

- 1.5 ml or 2.0 ml reaction tubes
- 96–99.8 % ethanol; non denatured or methylated
- ddH_2O

7 Product specifications

1. Starting material:
   - Stool samples from different origins (200–400 mg)

2. Time for isolation:
   - Approximately 30–45 minutes depending on the kind of application

3. Binding capacity and typical yield:
   - Not determined. Sufficient DNA for enzymatic amplification reactions.

   The extracted gDNA can be used for a wide range of different molecular biology applications.

8 Initial steps before starting

- Heat thermal mixer or water bath to the required temperature (95 °C and 70 °C)
- Add the indicated amount of absolute ethanol to **Washing Solution HS (conc.)**, mix thoroughly and store as described above. Always keep the bottle firmly closed.

845-KS-7010010	Add 5 ml ethanol to 5 ml Washing Solution HS (conc.).
845-KS-7010050	Add 20 ml ethanol to 20 ml Washing Solution HS (conc.).

- Add the indicated amount of absolute ethanol to **Washing Solution MS (conc.)**, mix thoroughly and store as described above. Always keep the bottle firmly closed.

845-KS-7010010	Add 7 ml ethanol to 3 ml Washing Solution MS (conc.).
845-KS-7010050	Add 35 ml ethanol to 15 ml Washing Solution MS (conc.).

- Add the indicated amount of ddH_2O to the vial of **Proteinase K**, mix thoroughly and store as described above.

845-KS-7010010	Add 0.3 ml ddH_2O to lyophilized Proteinase K.
845-KS-7010050	Add 1.5 ml ddH_2O to lyophilized Proteinase K.

- Centrifugation steps should be carried out at room temperature.
- Avoid freezing and thawing of starting material

9 Protocol 1: Isolation of bacterial DNA from stool samples

1. Weigh **200-400 mg of stool sample** (fresh or frozen) into a 2.0 ml safe-lock tube.

NOTE

If the sample is liquid, pipette 200-400 µl into the safe-lock tube. Cut the end of the pipette tip to make pipetting easier.

2. Add **1 ml Lysis Solution SLS** to each stool sample. Vortex vigorously for 1 minute to get a homogenous suspension.
3. Incubate the sample for 15 minutes at 95 °C in thermal mixer under continuous shaking at 900 rpm

NOTE

The incubation step at 95 °C will maximize the amount of bacterial DNA because of a very efficient destruction of the cell walls of e.g. gram+-bacteria.

4. Transfer **650 µl** of the **sample volume** onto the Prefilter located in a 2.0 ml Receiver Tube. Centrifuge at 10.000 x g (~12.000 rpm) for 2 minutes. Remove and discard the Prefilter. Transfer the filtrate into a 1.5 ml reaction tube (not included in the kit).

NOTE

If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.

5. Add **25 µl Proteinase K** to the sample and mix it shortly. Incubate the sample for 20 minutes at 70 °C in a thermal mixer under continuous shaking at 900 rpm
6. Add **300 µl Binding Solution SBS** to the lysed sample and mix by pipetting up and down several times. Apply **650 µl** of the **sample** to the Spin Filter located in a 2.0 ml Receiver Tube and centrifuge at 10.000 x g (~12.000 rpm) for 2 minutes.

NOTE

If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.

7. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube and apply the residual sample to the Spin Filter. Centrifuge at 10.000 x g (~12.000 rpm) for 2 minutes. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.
8. Add **600 µl Washing Solution HS** and centrifuge at 10.000 x g (~12.000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.
9. Add **750 µl Washing Solution MS** and centrifuge at 10.000 x g (~12.000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.
10. Centrifuge at max. speed for 2 minutes to remove all traces of ethanol. Discard the 2.0 ml Receiver Tube.
11. Place the Spin Filter into a 1.5 ml reaction tube and add **100–200 µl Elution Buffer** onto the center of the Spin Filter. Incubate at room temperature for 1 minute. Centrifuge at 6.000 x g (~8.000 rpm) for 1 minute. Dividing the final elution volume in two equal volumes of Elution Buffer increases the final concentration of DNA in the first elution step, but not the yield of eluted DNA.

NOTE

The DNA can be eluted with a lower or higher volume of **Elution Buffer** (depends on the expected yield of genomic DNA). Elution with lower volumes of **Elution Buffer** increases the final concentration of DNA. Store the extracted DNA at 4–8 °C. For long time storage placing at -22 °C to -18 °C is recommended.

10 Protocol 2: Isolation of host DNA from stool samples

1. Weigh **200-400 mg of stool sample** (fresh or frozen) into a 2.0 ml safe-lock tube.

NOTE

If the sample is liquid, pipette 200-400 µl into the safe-lock tube. Cut the end of the pipette tip to make pipetting easier.

2. Add **1 ml Lysis Solution SLS** to each stool sample. Vortex vigorously for 1 minute to get a homogenous suspension.
3. Transfer **650 µl** of the **sample volume** onto the Prefilter located in a 2.0 ml Receiver Tube. Centrifuge at 10.000 x g (~12.000 rpm) for 2 minutes. Remove and discard the Prefilter. Transfer the filtrate into a 1.5 ml reaction tube (not included in the kit).

NOTE

If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.

4. Add **25 µl Proteinase K** to the sample and mix it shortly. Incubate the sample for 20 minutes at 70 °C in a thermal mixer under continuous shaking at 900 rpm
5. Add **300 µl Binding Solution SBS** to the lysed sample and mix by pipetting up and down several times. Apply **700 µl** of the **sample** to the Spin Filter located in a 2.0 ml Receiver Tube and centrifuge at 10.000 x g (~12.000 rpm) for 2 minutes.

NOTE

If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.

6. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube and apply the residual sample to the Spin Filter. Centrifuge at 10.000 x g (~12.000 rpm) for 2 minutes. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.

7. Add **600 µl Washing Solution HS** and centrifuge at 10.000 x g (~12.000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.
8. Add **750 µl Washing Solution MS** and centrifuge at 10.000 x g (~12.000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.
9. Centrifuge at max. speed for 2 minutes to remove all traces of ethanol. Discard the 2.0 ml Receiver Tube.
10. Place the Spin Filter into a 1.5 ml reaction tube and add **100–200 µl Elution Buffer** onto the center of the Spin Filter. Incubate at room temperature for 1 minute. Centrifuge at 6.000 x g (~8.000 rpm) for 1 minute. Dividing the final elution volume in two equal volumes of Elution Buffer increases the final concentration of DNA in the first elution step, but not the yield of eluted DNA.

NOTE

The DNA can be eluted with a lower or higher volume of **Elution Buffer** (depends on the expected yield of genomic DNA). Elution with lower volumes of **Elution Buffer** increases the final concentration of DNA. Store the extracted DNA at 4–8 °C. For long time storage placing at -22 °C to -18 °C is recommended.

11 Troubleshooting

Problem / probable cause	Comments and suggestions
Clogged Spin Filter	
Insufficient lysis and/or too much starting material	Increase lysis time. Increase centrifugation speed. After lysis centrifuge the lysate to pellet unlysed material.
Low amount of extracted DNA	
Insufficient lysis	Increase lysis time. Reduce amount of starting material. Overloading of Spin Filter reduces yield!
Incomplete elution	Prolong the incubation time with **Elution Buffer** to 5 minutes or repeat elution step once again. Take higher volume on **Elution Buffer.**
Insufficient mixing with Binding Solution SBS	Mix sample with **Binding Solution SBS** by pipetting or vortexing before transferring the sample to the **Spin Filter**.
Problems with down-stream application, e.g. ligation	
Contamination with salt components	Wash the **Spin Filter** as described in the manual.
Contamination of the final DNA with ethanol	Keep the given centrifugation time, extend it if necessary (test the smell).

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