Instructions for Use
Life Science Kits & Assays

innuSPEED Soil DNA Kit 2.0

Innuscreen GmbH

Order No.:
845-KS-1580010 10 reactions
845-KS-1580050 50 reactions

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1 Introduction

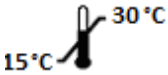
1.1 Intended use

The **innuSPEED Soil DNA Kit 2.0** has been designed as a tool for extracting environmental DNA (eDNA) from soil samples. The kit is based on a patented DNA extraction technology and combines an initial very efficient homogenization step with the subsequent binding of the DNA on a spin filter. After washing steps, the bound DNA is eluted using a low salt elution buffer. The extracted DNA can be used for different downstream application like PCR, qPCR, Digital PCR or Nanopore Sequencing.

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 uses the following warning and information symbols as well as the shown methodology:

Symbol	Information
	**REF** Catalogue number.
	**Content** Contains sufficient reagents for <N> reactions.
	**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 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 kit 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).
- Working steps are numbered.

2 Safety precautions

NOTE
Read through this chapter carefully before use 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 personal in a laboratory environment!

If the buffer bottles are damaged or leaking, wear gloves and protective goggles when discarding the bottles to avoid any injuries. IST Innuscreen GmbH has not tested the liquid waste generated during use of the kit for potential residual infectious components. This case is highly unlikely but cannot be excluded completely. 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.

All components of the **innuSPEED Soil DNA Kit 2.0** 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.

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

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 kit was 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 **innuSPEED Soil DNA Kit 2.0** or other products, please do not hesitate to contact us. For technical support or further information in Germany please contact info.innu@ist-ag.com. 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 only 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 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-1580010	845-KS-1580050
Lysis Tube B 2.0	10	50
Lysis Solution MA	9 ml	45 ml
Precipitation Buffer P	2 ml	6 ml
Binding Solution BL	10 ml	50 ml
Binding Solution RBS	3 ml	11 ml
Washing Solution C	8 ml	50 ml
Washing Solution BS (conc.)	1 ml	5 ml
Elution Buffer	2 x 2 ml	12 ml
Spin Filter	20	2 x 50
Receiver Tubes (2.0 ml)	20	2 x 50
Elution Tubes (1.5 ml)	20	2 x 50
Manual	1	1

6.2 Components not included in the kit

- 2.0 ml reaction tubes
- 96-99.8% ethanol (molecular biology grade, undenatured)
- 80% ethanol (molecular biology grade, undenatured)

SpeedMill (homogenizer from Analytik Jena GmbH) or other type of homogenizer like Precellys, Fastprep, Bead Raptor etc.

7 Product Specifications

1. Starting material
    - up to 400 mg soil sample

NOTE
Avoid freezing and thawing of starting material!

8 Initial steps before starting

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

845-KS-1580010	Add 9 ml ethanol to 1 ml Washing Solution BS (conc.).
845-KS-1580050	Add 45 ml ethanol to 5 ml Washing Solution BS (conc.).

- Centrifugation steps should be carried out at room temperature.

9 DNA extraction from soil samples

9.1 Homogenization process using commercially available Homogenizers (e.g. SpeedMill, Precellys, Fastprep, Bead Raptor etc.)

1. Transfer max. 400 mg soil starting material into **a Lysis Tube B 2.0**.
2. Add **800 µl Lysis Solution MA**, mix shortly by pulsed vortexing for 5 sec.
3. Place the **Lysis Tube B 2.0** in the Homogenizer and start the homogenization for 1 min.

NOTE
The homogenization process can be changed and optimized depending on the used homogenizer. The optimal duration and intensity of homogenization depends on kind of homogenizer used.

9.2 DNA extraction

4. Remove the **Lysis Tube B 2.0** from the Homogenizer and centrifuge the Lysis Tube B 2.0 at max. speed for 7 min.
5. Open the Lysis Tube B 2.0 and transfer the supernatant carefully into a new 2.0 ml reaction tube. Add **100 µl Precipitation Buffer P** and vortex shortly for 5 s. Leave the tube for 3 min.
6. Centrifuge at maximum speed for 5min. Carefully transfer the **supernatant** into a new 2.0 ml reaction tube (determine the volume of the supernatant).

NOTE
Avoid carry-over of pellet-material. If the transferred supernatant contains residual pellet components, centrifuge the sample again for 2 min. at max. speed and transfer the clear supernatant into a new 2.0 ml tube.

7. Add an equal volume **Binding Solution BL** to the sample, mix by pipetting up and down several times. It is important that the sample and the **Binding Solution BL** are mixed thoroughly to get a homogeneous solution.

8. Apply **750 µl of the sample** onto a Spin Filter located in a 2.0 ml Receiver Tube and centrifuge at 11.000 x g for 1 min. Discard the filtrate and reuse the Receiver Tube. Place the Spin Filter back into the Receiver Tube.
9. Apply the **residual sample** and centrifuge at 11,000 x g for 1 min. Discard the filtrate and reuse the Receiver Tube. Place the Spin Filter back into the 2.0 ml Receiver Tube.
10. Add **700 µl Washing Solution C** and incubate 1 min at room temperature. Centrifuge at 11,000 x g for 1 min. Discard the filtrate and reuse the Receiver Tube. Place the Spin Filter back into the 2.0 ml Receiver Tube.
11. Add **700 Washing Solution BS** and centrifuge at 11,000 x g for 1 min. Discard the filtrate and reuse the Receiver Tube. Place the Spin Filter back into the 2.0 ml Receiver Tube.
12. Add **400 µl absolute ethanol** and centrifuge at max. speed for 3 minutes to remove all traces of ethanol. Carefully remove the spin filter and place the Spin Filter in a 1.5 ml Elution Tube. Discard the 2.0 ml Receiver Tube.
13. Add **50 - 100 µl pre-heated Elution Buffer** (70°C). Incubate at room temperature for 2 minutes. Centrifuge at 11,000 x g for 1 min.

NOTE

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

2. Extracted DNA is pure and ready to use for downstream application.

3. In rare cases, the eluate may be discolored. If the eluate is discolored, it is recommended to use the cleanup protocol

9.3 Cleanup Protocol (to remove optionally the impurities from eluate)

1. Add **200 µl Binding Solution RBS** to the eluate. Mix by pipetting up and down several times. Avoid thereby formation of foam.
2. Apply the sample to in a Spin Filter located in a 2.0 ml Receiver Tube and centrifuge at 11,000 x g for1 minute. Discard the filtrate and reuse the Receiver Tube. Place the Spin Filter back into the Receiver Tube.
3. Add **750 µl 80% ethanol** and centrifuge at 11.000 x g for 1 min. Discard the filtrate and reuse the Receiver Tube. Place the Spin Filter back into the 2.0 ml Receiver Tube.
4. Repeat step 3 once again.
5. Centrifuge at maximum speed for 3 min. to remove all traces of ethanol. Discard the Receiver Tube and place the Spin Filter in a 1.5 ml Elution Tube and add **50 - 100 µl pre-heated Elution Buffer** (70°C). Incubate at room temperature for 2 minutes. Centrifuge at 11,000 x g for 1 min.

NOTE
Extracted DNA is pure and ready to use for downstream application.

10 Troubleshooting

Problem / probable cause	Comments and suggestions
Clogged Spin Filter	
Insufficient lysis and/or too much starting material	Reduce amount of starting material.
Low amount of extracted DNA	
Insufficient homogenization	Increase homogenization time and/or speed.
Insufficient lysis	Reduce amount of starting material. Overloading of spin filter reduces yield!
Incomplete elution	Prolong the incubation time with Elution Buffer to 5 min. or repeat elution step. Take a higher volume of Elution Buffer.
Insufficient mixing with Binding Solution	Mix sample with Binding Solution BL by pipetting or by vortexing prior to transfer of the sample onto the spin filter.
Low concentration of extracted DNA	
Too much Elution Buffer	Elute DNA in a lower volume of Elution Buffer.
Eluates are colored	
Incorrect washing steps	Perform cleanup protocol.

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