Instructions for Use
Life Science Kits & Assays

innuSPEED Soil DNA Kit
Order No.:
845-KS-1580010 10 reactions
845-KS-1580050 50 reactions
845-KS-1580250 250 reactions
Publication No.: HB_KS-1580_e_120116

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It needs not necessarily agree with future versions. Subject to change!

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1 Safety precautions
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.

2 Storage conditions
The innuSPEED Soil DNA Kit should be stored dry, at room temperature (14–25 °C) and is stable for at least 12 months under these conditions. Before every use make sure that all components have room temperature. If there are any precipitates within the provided solutions solve these precipitates by careful warming.

3 Function testing and technical assistance
The Analytik Jena AG guarantees the correct function of the kit for applications as described in the manual. The components of each innuSPEED Soil DNA Kit were tested by isolation of total DNA from soil samples and subsequent RAPD PCR.

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 or other Analytik Jena AG products, please do not hesitate to contact us. For technical support or further information in Germany please dial +49 36 41 / 77 94 00. For other countries please contact your local distributor.

4 Product use and warranty
The user is responsible to validate the performance of the Analytik Jena AG kits for any particular use, since the performance characteristics of our kits have not been validated for any specific application. Analytik Jena AG 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 equivalents in other countries.

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

Note
For research use only!
# 5 Kit components

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**Important**
All components are stored at room temperature.

<table>
<thead>
<tr>
<th></th>
<th>10 extractions</th>
<th>50 extractions</th>
<th>250 extractions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lysis Tube B</strong></td>
<td>10</td>
<td>50</td>
<td>250</td>
</tr>
<tr>
<td><strong>Lysis Solution ELS</strong></td>
<td>8 ml</td>
<td>35 ml</td>
<td>160 ml</td>
</tr>
<tr>
<td><strong>Binding Solution TBS</strong></td>
<td>5 ml</td>
<td>18 ml</td>
<td>90 ml</td>
</tr>
<tr>
<td><strong>Binding Solution RBS</strong></td>
<td>5 ml</td>
<td>24 ml</td>
<td>110 ml</td>
</tr>
<tr>
<td><strong>Washing Solution RL</strong></td>
<td>15 ml</td>
<td>75 ml</td>
<td>2 x 175 ml</td>
</tr>
<tr>
<td><strong>Washing Solution MS</strong></td>
<td>6 ml (final volume 20 ml)</td>
<td>24 ml (final volume 80 ml)</td>
<td>2 x 54 ml (final volume 2 x 180 ml)</td>
</tr>
<tr>
<td><strong>Elution Buffer</strong></td>
<td>2 ml</td>
<td>15 ml</td>
<td>60 ml</td>
</tr>
<tr>
<td><strong>Spin Filter (vanilla)</strong></td>
<td>20</td>
<td>2 x 50</td>
<td>10 x 50</td>
</tr>
<tr>
<td><strong>Receiver Tubes (2.0 ml)</strong></td>
<td>50</td>
<td>5 x 50</td>
<td>25 x 50</td>
</tr>
<tr>
<td><strong>Elution Tubes (1.5 ml)</strong></td>
<td>20</td>
<td>2 x 50</td>
<td>10 x 50</td>
</tr>
<tr>
<td><strong>Manual</strong></td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

**Initial steps**

- Add 14 ml of 96-99.8 % ethanol to the bottle Washing Solution MS, mix thoroughly and keep the bottle always firmly closed!
- Add 56 ml of 96-99.8 % ethanol to the bottle Washing Solution MS, mix thoroughly and keep the bottle always firmly closed!
- Add 126 ml of 96-99.8 % ethanol to each bottle Washing Solution MS, mix thoroughly and keep the bottle always firmly closed!
6 **Recommended steps before starting**

- Heat thermal mixer or water bath at 95 °C – 98 °C
- Ensure that the Washing Solution MS has been prepared according to the instruction (→ "Kit components" p. 4)
- Centrifugation steps should be carried out at room temperature
- Avoid freezing and thawing of starting material

7 **Components not included in the kit**

- 1.5 ml reaction tubes
- 96-99.8 % ethanol
- SpeedMill (homogenizer from Analytik Jena AG) or other type of homogenizer
8 General procedure for DNA extraction

- Lysis and homogenization of starting material
- Pre-binding and final binding of DNA onto Spin Filter (vanilla)
- Pre-washing and final washing of the bound DNA
- Pre-elution and final elution of DNA
Protocol 1: DNA extraction from soil samples using SpeedMill

Important
Please note that up to 100 mg of soil material can be processed.

A. Homogenization process using SpeedMill
1. Transfer max. 100 mg soil starting material into a Lysis Tube B.
2. Add 600 µl Lysis Solution ELS, mix virgourously by pulsed vortexing for 10 sec and incubate at 95 °C – 98 °C for 20 minutes.
   
   Note: We recommend to use a shaking platform (thermal mixer, water bath or another rocking platform) for a continuous shaking of the sample. Alternatively, vortex the sample 3-4 times during the incubation. No shaking will reduce the lysis efficiency.

3. Place the Lysis Tube B in the SpeedMill and start the homogenization process for 4 minutes.
   
   Note: Optionally the homogenization can be changed and optimized regarding on the special application or starting material

B. Extraction procedure

Remove the Lysis Tube B from the SpeedMill and centrifuge the Lysis Tube B at max. speed for 5 minutes.

Open the Lysis Tube B and transfer 300 µl of the supernatant very carefully into a new 1.5 ml reaction tube.

Important note: Avoid carryover of soil material. If the transferred supernatant contains residual sample components, centrifuge the sample again for 2 minutes at max. speed and transfer the clean supernatant into a new 1.5 ml reaction tube.

1. Pre-binding

Add 300 µl of Binding Solution TBS to the sample, mix by vortexing or pipetting up and down several times. It is important that the sample and the Binding Solution TBS are mixed vigorously to get a homogeneous solution.

Apply the sample onto a Spin Filter (vanilla) located in a 2.0 ml Receiver Tube. Close the cap 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.

Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.

2. Pre-washing

Open the Spin Filter and add **500 µl Washing Solution RL**, close the cap and centrifuge at 10,000 x g (~12,000 rpm) for 1 minute. Discard the filtrate and place the Spin Filter back into the 2.0 ml Receiver Tube.

Repeat the washing step once again. Discard the filtrate and place the Spin Filter back into the 2.0 ml Receiver Tube.

Open the Spin Filter and add **700 µl Washing Solution MS**, close the cap and centrifuge at 10,000 x g (~12,000 rpm) for 1 minute. Discard the 2.0 ml Receiver Tube with the filtrate.

3. Pre-elution

Place the Spin Filter into a 1.5 ml Elution Tube. Carefully open the cap of the Spin Filter and add **100 µl Elution Buffer**. Incubate at room temperature for 2 minutes. Centrifuge at 5,000 x g for 1 minute. Remove and discard the Spin Filter.

**The Elution Tube contains the sample. Do not discard the Elution Tube!**

4. Final binding

Add **300 µl Washing Solution RL and 400 µl Binding Solution RBS** to the sample, mix by vortexing or by pipetting up and down several times. It is important that the sample and the Binding Solution RBS are mixed vigorously to get a homogeneous solution.

Apply the sample onto a new Spin Filter located in a 2.0 ml Receiver Tube. Close the cap and 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.
innuSPEED Soil DNA Kit

Protocol: DNA isolation from soil samples

Recommended steps before starting
- Heat thermal mixer or water bath to 95 – 98 °C
- Prepare Washing Solution MS according to the instruction

1. Starting material
   - Soil material
   - Max. 100 mg

2. Lysis
   - Add soil material to Lysis Tube B
   - Add 600 µl ELS
   - Vortex: 10 sec
   - Incubation: 20 min @ 95 – 98 °C

3. Homogenization
   - Add Lysis Tubes B to SpeedMill
   - Homogenize: 4 min
   - Centrifuge: max speed, 5 min
   - 300 µl supernatant to 1.5 ml tube

4. Pre-binding of DNA
   - Add 300 µl TBS and mix
   - Spin Filter to Receiver Tube
   - Add sample to Spin Filter
   - 10,000 x g (~12,000 rpm): 2 min

5. Pre-washing
   - Add 500 µl RL to Spin Filter
   - 10,000 x g (~12,000 rpm): 1 min
   - Repeat washing RL
   - Add 700 µl MS to Spin Filter
   - 10,000 x g (~12,000 rpm): 1 min

6. Pre-elution
   - Spin Filter to an Elution Tube
   - Add 100 µl Elution Buffer
   - Incubation: 2 min @ RT
   - 5,000 x g: 1 min
   - Discard Spin Filter
7. Final Binding
   - Add 300 µl RL and 400 µl RBS to the filtrate
   - Vortex
   - Add Spin Filter to Receiver Tube
   - Add sample to Spin Filter
   - 10,000 x g (~12,000 rpm): 2 min

8. Final washing
   - Add 650 µl MS to Spin Filter
   - 10,000 x g (~12,000 rpm): 1 min

9. Remove Ethanol
   - Discard filtrate
   - Spin Filter to Receiver Tube
   - Centrifuge: max speed, 2 min

10. Final elution
    - Spin Filter to an Elution Tube
    - Add 80 µl Elution Buffer
    - Incubation: 3 min @ RT
    - 6,000 x g (~8,000 rpm): 1 min

Order No.: 845-KS-1580010  10 reactions
           845-KS-1580050  50 reactions
           845-KS-1580250  250 reactions

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Fax  +49 (0) 36 41 / 77-76 77 76
5. Final washing

Open the Spin Filter and add **650 μl Washing MS**, close the cap 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.

Centrifuge at max. speed for 2 minutes to remove all traces of ethanol. Discard the 2.0 ml Receiver Tube.

6. Final elution

Place the Spin Filter into a new 1.5 ml Elution Tube. Carefully open the cap of the Spin Filter and add **80 μl Elution Buffer**. Incubate at room temperature for 3 minutes. Centrifuge at 6,000 x g (~8,000 rpm) for 1 minute.

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**Note**

The DNA can be eluted with a lower or a higher volume of Elution Buffer (depends 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. For long time storage placing at −20 °C is recommended.
10 Protocol 2: DNA extraction from soil samples using other homogenizers

Important
Please note that up to 250 mg of soil material can be processed.

For the homogenization of tissue samples it is possible to use commercially available homogenizer which work with 2.0 ml “Grinding Tubes” e.g. the homogenizer “Precellys” or the homogenizer “FastPrep”!

A. Homogenization process using other homogenizers

1. Transfer 100 – 250 mg soil starting material into a Lysis Tube B.
2. Add 600 µl Lysis Solution ELS, mix virgourously by pulsed vortexing for 10 sec and incubate at 95 °C – 98 °C for 20 minutes.
   
   **Note:** We recommend to use a shaking platform (thermal mixer, water bath or another rocking platform) for a continuous shaking of the sample. Alternatively, vortex the sample 3-4 times during the incubation. No shaking will reduce the lysis efficiency.

3. Place the Lysis Tube B in a homogenizer and start the homogenizing process according to the instruction manual of the homogenizer by using the recommended parameters for the homogenization of soil samples.
   
   **Note:** The time for homogenization and the power of homogenization depends on the kind of homogenizer used. Please find the individual parameter for the specific application!

B. Extraction procedure

Remove the Lysis Tube B from the homogenizer and centrifugue the Lysis Tube B at max. speed for 5 minutes.

Open the Lysis Tube B and transfer 300 µl of the supernatant very carefully into a new 1.5 ml reaction tube.

**Important note:** Avoid carryover of soil material. If the transferred supernatant contains residual sample components, centrifugue the sample again for 2 minutes at max. speed and transfer the clean supernatant into a new 1.5 ml reaction tube.
1. Pre-binding
Add 300 µl of Binding Solution TBS to the sample, mix by vortexing or pipetting up and down several times. It is important that the sample and the Binding Solution TBS are mixed vigorously to get a homogeneous solution.

Apply the sample onto a Spin Filter (vanilla) located in a 2.0 ml Receiver Tube. Close the cap 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.

Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.

2. Pre-washing
Open the Spin Filter and add 500 µl Washing Solution RL, close the cap and centrifuge at 10,000 x g (~12,000 rpm) for 1 minute. Discard the filtrate and place the Spin Filter back into the 2.0 ml Receiver Tube.

Repeat the Washing step once again. Discard the filtrate and place the Spin Filter back into the 2.0 ml Receiver Tube.

Open the Spin Filter and add 700 µl Washing Solution MS, close the cap and centrifuge at 10,000 x g (~12,000 rpm) for 1 minute. Discard the 2.0 ml Receiver Tube with the filtrate.

3. Pre-elution
Place the Spin Filter into a 1.5 ml Elution Tube. Carefully open the cap of the Spin Filter and add 100 µl Elution Buffer. Incubate at room temperature for 2 minutes. Centrifuge at 5,000 x g for 1 minute. Remove and discard the Spin Filter.

**The Elution Tube contains the sample. Do not discard the Elution Tube!**

4. Final binding
Add 300 µl Washing Solution RL and 400 µl Binding Solution RBS to the sample, mix by vortexing or by pipetting up and down several times. It is important that the sample and the Binding
Solution RBS are mixed vigorously to get a homogeneous solution.

Apply the sample onto a new Spin Filter located in a 2.0 ml Receiver Tube. Close the cap and 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.

5. Final washing

Open the Spin Filter and add 650 µl Washing MS, close the cap 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.

Centrifuge at max. speed for 2 minutes to remove all traces of ethanol. Discard the 2.0 ml Receiver Tube.

6. Final elution

Place the Spin Filter into a new 1.5 ml Elution Tube. Carefully open the cap of the Spin Filter and add 80 µl Elution Buffer. Incubate at room temperature for 3 minutes. Centrifuge at 6,000 x g (~8,000 rpm) for 1 minute.

Note

The DNA can be eluted with a lower or a higher volume of Elution Buffer (depends 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. For long time storage placing at −20 °C is recommended.
# 11 Troubleshooting

<table>
<thead>
<tr>
<th>Problem / probable cause</th>
<th>Comments and suggestions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clogged Spin Filter</strong></td>
<td>Increase lysis time.</td>
</tr>
<tr>
<td>• Insufficient lysis and/or too much starting material</td>
<td>Increase centrifugation speed. After lysis centrifuge the lysate to pellet unlysed material. Reduce amount of starting material.</td>
</tr>
<tr>
<td><strong>Low amount of extracted DNA</strong></td>
<td>Increase homogenization time and/or the speed of homogenization (using either homogenizer)</td>
</tr>
<tr>
<td>• Insufficient homogenization</td>
<td>Increase lysis time at 95 – 98 °C. Reduce amount of starting material. Overloading of Spin Filter reduces yield!</td>
</tr>
<tr>
<td>• Insufficient lysis</td>
<td>Prolong the incubation time with Elution Buffer to 5 minutes or repeat elution step once again. Take a higher volume of Elution Buffer.</td>
</tr>
<tr>
<td>• Incomplete elution</td>
<td>Mix sample with Binding Solution TBS by pipetting or by vortexing prior to transfer of the sample onto the Spin Filter.</td>
</tr>
<tr>
<td>• Insufficient mixing with Binding Solution TBS</td>
<td></td>
</tr>
<tr>
<td><strong>Low concentration of extracted DNA</strong></td>
<td>Elute DNA with a lower volume of Elution Buffer.</td>
</tr>
<tr>
<td>• Too much Elution Buffer</td>
<td></td>
</tr>
<tr>
<td><strong>Eluates are yellow</strong></td>
<td>Perform all washing steps exactly as described in the manual</td>
</tr>
<tr>
<td>• Incorrect washing steps or wrong starting material</td>
<td></td>
</tr>
</tbody>
</table>