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Life Science unlimited

Manual

innuSPEED Tissue DNA Kit

Order No.:

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Contents

1 Safety precautions 3

2 Storage conditions 3

3 Function testing and technical assistance 3

4 Product use and warranty 3

5 Kit components 4

6 Recommended steps before starting 5

7 Components not included in the kit 5

8 General procedure for DNA extraction 6

9 Protocol 1: DNA extraction from tissue material (up to 50 mg), rodent tails or other cartilages using SpeedMill 7

10 Protocol 2: DNA extraction from tissue material (up to 50 mg), rodent tails or other cartilages using other homogenizers 9

11 Troubleshooting 9

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 Tissue 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 Tissue DNA Kit were tested by isolation of genomic DNA from tissue material and subsequent target-amplification.

We reserve the right to change or modify our products to enhance there performance and design. If you have any questions or problems regarding any aspects of the innuSPEED Tissue 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

Important

Store lyophilized Proteinase K at +4 °C. Store the dissolved Proteinase K as described below! All other components are stored at room temperature.

	10 extractions	**50 extractions**	**250 extractions**
Lysis Tube P	10	50	5 x 50
Lysis Solution TLS	2 x 2 ml	15 ml	70 ml
Precipitation Buffer	1 ml	3 x 2 ml	25 ml
Proteinase K	for 0.3 ml working solution	for 1.5 ml working solution	for 5 x 1.5 ml working solution
Washing Solution HS	3 ml (final volume 6 ml)	15 ml (final volume 30 ml)	70 ml (final volume 140 ml)
Washing Solution MS	3 ml (final volume 10 ml)	15 ml (final volume 50 ml)	60 ml (final volume 200 ml)
Elution Buffer	2 x 2 ml	25 ml	2 x 60 ml
Spin Filter (blue)	10	50	5 x 50
Receiver Tubes (2.0 ml)	40	4 x 50	20 x 50
Elution Tubes (1.5 ml)	10	50	5 x 50
Manual	1	1	1
Initial steps	• Add 3 ml of 96-99.8 % ethanol to the bottle Washing Solution HS, mix thoroughly and keep the bottle always firmly closed! • Add 7 ml of 96-99.8 % ethanol to the bottle Washing Solution MS, mix thoroughly and keep the bottle always firmly closed! • Dissolve Proteinase K by addition of 0.3 ml of ddH_2O, mix thoroughly and store as described below	• Add 15 ml of 96-99.8 % ethanol to the bottle Washing Solution HS, mix thoroughly and keep the bottle always firmly closed! • Add 35 ml of 96-99.8 % ethanol to the bottle Washing Solution MS, mix thoroughly and keep the bottle always firmly closed! • Dissolve Proteinase K by addition of 1.5 ml of ddH_2O, mix thoroughly and store as described below	• Add 70 ml of 96-99.8 % ethanol to the bottle Washing Solution HS, mix thoroughly and keep the bottle always firmly closed! • Add 140 ml of 96-99.8 % ethanol to the bottle Washing Solution MS, mix thoroughly and keep the bottle always firmly closed! • Dissolve Proteinase K by addition of 1.5 ml of ddH_2O, mix thoroughly and store as described below

Important

Store dissolved Proteinase K at –20 °C, but repeated freezing and thawing will reduce the activity dramatically. Dividing the Proteinase K into aliquots and storage at – 20 °C is recommended.

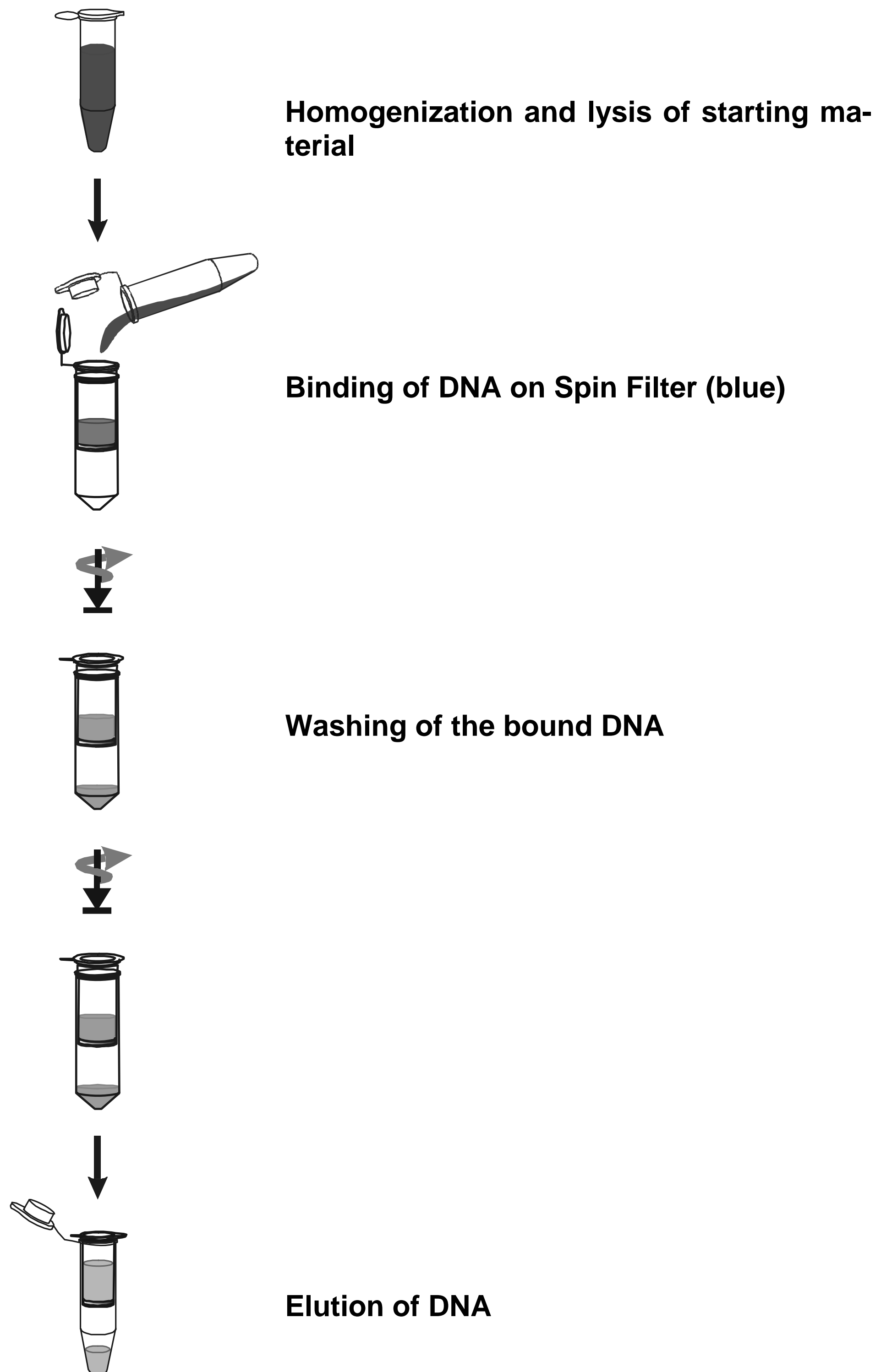
6 Recommended steps before starting

- Heat thermal mixer or water bath at 50 °C
- Ensure that the Washing Solution HS, Washing Solution MS and Proteinase K have 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

- RNase A (100 mg/ml); optional
- 1.5 ml tubes
- 2.0 ml tubes; optional
- 70 % ethanol
- 96-99.8 % ethanol
- ddH_2O
- SpeedMill P12 (homogenizer from Analytik Jena AG) or other type of homogenizer

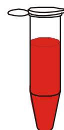
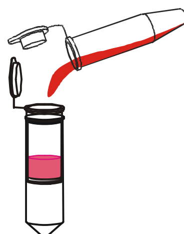
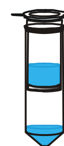
8 General procedure for DNA extraction



✂ Cut at the scattered line and laminate the card for a more convenient handling on the table top ✂

innuSPEED Tissue DNA Kit

Protocol: DNA isolation from tissue samples

Recommended steps before starting		▪ Heat thermal mixer or water bath (50 °C) ▪ Prepare Washing Solution HS, Washing Solution MS and Proteinase K according to the instruction
1. Starting material	▪ Tissue, rodent tail or other cartilage material	▪ Up to 50 mg
2. Homogenization		▪ Cut tissue material (small pieces) ▪ Add cut material to Lysis Tube P ▪ Add 50 µl H_2O ▪ Add Lysis Tubes P to SpeedMill ▪ Homogenize: 30 sec – 2 x 2 min
3. Lysis		▪ Add 250 µl TLS <u>and</u> 25 µl PK ▪ Vortex: 5 sec ▪ Incubation: 50 °C, 30 min
4. Optional: RNA removal		▪ 100 mg/ml RNase A; vortex ▪ Incubation: 5 min @ RT
5. Removing of proteins		▪ Add 75 µl Precipitation Buffer ▪ Vortex: 10 sec ▪ Zentrifuge: max speed, 3 min ▪ Add supernatant to a 1.5 ml tube
6. Binding of DNA		▪ Add 500 µl ethanol (70 %) ▪ Add Spin Filter to Receiver Tube ▪ Add sample to Spin Filter ▪ 10.000 x g (~12.000 rpm): 2 min
7. Washing New Receiver Tubes		▪ Add 500 µl HS ▪ 10.000 x g (~12.000 rpm): 1 min ▪ Add 700 µl MS ▪ 10.000 x g (~12.000 rpm): 1 min

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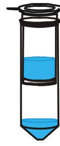
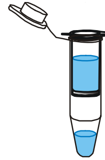
8. Remove Ethanol New Receiver Tubes			▪ Discard filtrate ▪ Add Spin Filter to Receiver Tube ▪ Centrifuge: max speed, 2 min
9. Elution			▪ Add Spin Filter to an Elution Tube ▪ Add 200 µl Elution Buffer ▪ Incubation: 1 min @ RT ▪ 6.000 x g (~8.000 rpm): 1 min

Order No.: 845-KS-1540010 10 reactions
845-KS-1540050 50 reactions
845-KS-1540250 250 reactions

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9 Protocol 1: DNA extraction from tissue material (up to 50 mg), rodent tails or other cartilages using SpeedMill P12

Important
Please note that up to 50 mg of tissue material can be processed.

A. Homogenization process using SpeedMill P12

1. Cut the starting material into small pieces.

   Note: Efficiency of homogenization process depends on the size of starting material.

   The following table shows some recommended sizes depending on different groups of tissue material for an optimal homogenization process.

Table 1: Recommended optimal size of starting material

Groups of tissue material	Optimal size for homogenization
Soft tissue material like: lung, kidney, brain, spleen, liver etc.	appr. 5 mm x 5 mm
Very hard material like: rodent tails or cartilage material	appr. 1 mm

   In general, smaller pieces of starting material are better for homogenization.

2. Transfer the cut starting material into the Lysis Tube P. Add **50 µl H_2O**.
3. Place the Lysis Tube P in the SpeedMill P12 and start the homogenization process. Time for processing depends on type of starting material.

   The following table 2 shows a short overview about processing time depending on different types of starting material.

Table 2: Recommended homogenization time

Groups of tissue material	Recomm. time for homogenization
Soft tissue material like: lung, kidney, brain, spleen, liver etc.	appr. 30 sec – 1 minute
Very hard material like: rodent tails or cartilage material	appr. 2 x 2 minutes
Other materials: e.g. insects like ticks	appr. 2 x 2 minutes

Note: If starting material is not homogenized, please increase the homogenization time.

B. Extraction procedure

1. After homogenization please check, that the starting material is completely disrupted. Open the Lysis Tube P and add **250 µl** of **Lysis Solution TLS** and **25 µl Proteinase K**, mix vigorously by pulsed vortexing for 5 sec. Incubate at 50 °C for appr. 30 minutes (longer incubation is also possible).

   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.

   To remove RNA from the sample (if necessary) add 4 µl of RNase A solution (100 mg/ml) to the filtrate, vortex shortly and incubate for 5 min at RT.

2. Add **75 µl Precipitation Buffer** to the Lysis Tube P and vortex vigorously for 10 sec. Centrifuge the Lysis Tube P at max. speed for 3 minutes. Carefully transfer the cleared supernatant into a 1.5 ml reaction tube (avoid carry over of precipitate).

3. Add **500 µl 70 % ethanol** to the supernatant and mix by pipetting up and down several times.

   Note: It is important that the sample and the ethanol are mixed vigorously to get a homogeneous solution.

4. Apply the sample to the Spin Filter (blue) 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.

5. Open the Spin Filter and add **500 µl Washing Solution HS**, 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.
6. 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. Place the Spin Filter into a new 2.0 ml Receiver Tube.
7. Centrifuge at max. speed for 2 minutes to remove all traces of ethanol. Discard the 2.0 ml Receiver Tube.
8. Place the Spin Filter into a 1.5 ml Elution Tube. Carefully open the cap of the Spin Filter and add **200 µl Elution Buffer**. Incubate at room temperature for 1 minute. Centrifuge at 6.000 x g (~8.000 rpm) for 1 minute. Repeat the elution step once again to increase the yield of extracted DNA.

Note

The DNA can be eluted with a lower or a 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 °C. For long time storage placing at –20 °C is recommended.

10 Protocol 2: DNA extraction from tissue material (up to 50 mg), rodent tails or other cartilages using other homogenizers

Important
Please note that up to 50 mg of tissue 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. Cut the starting material into small pieces.
2. Transfer the cut starting material into the Lysis Tube P. Add 50 µl H_2O. Place the Lysis Tube P in a homogenizer and start the homogenizing process according instruction manual of the homogenizer.

   Note: The time for homogenization and the power of homogenization depends on the kind of homogenizer used. Normally 20 sec-3 min are sufficient for complete disruption of tissue material. Furthermore, the efficiency of homogenization depends on kind of tissue material. Please find the individual parameter for the specific application!

   If starting material is not homogenized, please increase the homogenization time.

B. Extraction Procedure

1. After homogenization please check, that the starting material is completely disrupted. Open the Lysis Tube P and add **250 µl** of **Lysis Solution TLS** and **25 µl Proteinase K**, mix vigorously by pulsed vortexing for 5 sec. Incubate at 50 °C for appr. 30 min (longer incubation is also possible).

   Note: We recommend to use a shaking platform (thermal mixer, water bath or another rocking platform) for a continuous shaking of the sample. Optionally, vortex the sample 3-4 times during the lysis step. No shaking will reduce the lysis efficiency.

   To remove RNA from the sample (if necessary) add 4 µl of RNase A solution (100 mg/ml) after incubation at 50 °C to the filtrate, vortex shortly and incubate for 5 min at RT.

2. Add **75 µl Precipitation Buffer** to the Lysis Tube P and vortex vigorously for 10 sec. Centrifuge the Lysis Tube P at max. speed for 3 min. Carefully transfer the cleared supernatant into a 1.5 ml reaction tube (avoid carry over of precipitate).

3. Add **500 µl 70 % ethanol** to the supernatant and mix by pipetting up and down several times.

   Note: It is important that the sample and the ethanol are mixed vigorously to get a homogeneous solution.

4. Apply the sample to the Spin Filter (blue) 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.

5. Open the Spin Filter and add **500 µl Washing Solution HS**, 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.

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

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

8. Place the Spin Filter into a 1.5 ml Elution Tube. Carefully open the cap of the Spin Filter and add **200 µl Elution Buffer**. Incubate at room temperature for 1 minute. Centrifuge at 6.000 x g (~8.000 rpm) for 1 minute. Repeat the elution step once again to increase the yield of extracted DNA.

Note

The DNA can be eluted with a lower or a 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 °C. For long time storage placing at –20 °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. Reduce amount of starting material.
• Insufficient homogenization	Increase time for homogenization.
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 a higher volume of Elution Buffer.
• Insufficient mixing with 70 % ethanol	Mix sample with ethanol by pipetting or by vortexing prior to transfer of the sample onto the Spin Filter.
Degraded or sheared DNA	
• Incorrect storage of starting material	Ensure that the starting material is frozen immediately in liquid N_2 or in minimum at –20 °C and is stored continuously at –80 °C! Avoid thawing of the material.
• Old material	Old material often contains degraded DNA.
RNA contaminations of extracted DNA	RNase A digestion

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