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# Life Science unlimited

## Manual

# blackPREP Rodent Tail DNA Kit

**Order No.:**

| | |
|---|---|
| 845-BP-0010010 | 10 reactions |
| 845-BP-0010050 | 50 reactions |
| 845-BP-0010250 | 250 reactions |

Publication No.: HB_PB-0010_e_120116

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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

The blackPREP Rodent Tail DNA Kit is the first product of a new product line for specialized kits for isolation of DNA from different kinds of starting materials.

The blackPREP Rodent Tail DNA Kit is designed for isolation of genomic DNA from rodent tails. The protocol has been specially optimized to get a maximum yield and quality of genomic DNA from rodent tails.

The extraction procedure is based on a new patented technology for isolation of DNA from complex starting materials. The extraction procedure combines a very fast and efficient lysis step with the subsequent binding of genomic DNA on a spin filter surface. The spin filter bounded DNA is washed and the DNA is eluted using low salt buffer.

Because of the very efficient lysis of rodent tail the extraction process is finished within max. 3 hours.

# 2 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.

Use the kit once only! For skilled personal only! Don't eat or drink components of the kit!

# 3 Storage conditions

The blackPREP Rodent Tail 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.

# 4 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 blackPREP Rodent Tail DNA Kit were tested by isolation of genomic DNA from mouse tail sample and subsequent DNA analysis.

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 blackPREP Rodent Tail 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.

# 5 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!

# 6 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 Solution QPT** | 5 ml | 25 ml | 120 ml |
| **Binding Solution SBS** | 2 x 2 ml | 15 ml | 70 ml |
| **Proteinase K** | for 1 x 0.3 ml working solution | for 1 x 1.5 ml working solution | for 5 x 1.5 ml working solution |
| **Washing Solution MS** | 6 ml (final volume 20 ml) | 24 ml (final volume 80 ml) | 2 x 60 ml (final vol. 2 x 200 ml) |
| **Elution Buffer** | 2 x 2 ml | 25 ml | 110 ml |
| **Spin Filter (black)** | 10 | 50 | 5 x 50 |
| **Receiver Tubes** (2.0 ml) | 20 | 2 x 50 | 10 x 50 |
| **Manual** | 1 | 1 | 1 |
| **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!<br>• Dissolve Proteinase K by addition of 0.3 ml of $ddH_2O$, mix thoroughly and store as described below! | • Add 56 ml of 96-99.8 % ethanol to the bottle Washing Solution MS, mix thoroughly and keep the bottle always firmly closed!<br>• Dissolve Proteinase K by addition of 1.5 ml of $ddH_2O$, mix thoroughly and store as described below! | • Add 140 ml of 96-99.8 % ethanol to the bottle Washing Solution MS, mix thoroughly and keep the bottle always firmly closed!<br>• 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 of the Proteinase K into aliquots and storage at –20 °C is recommended.

# 7 Recommended steps before starting

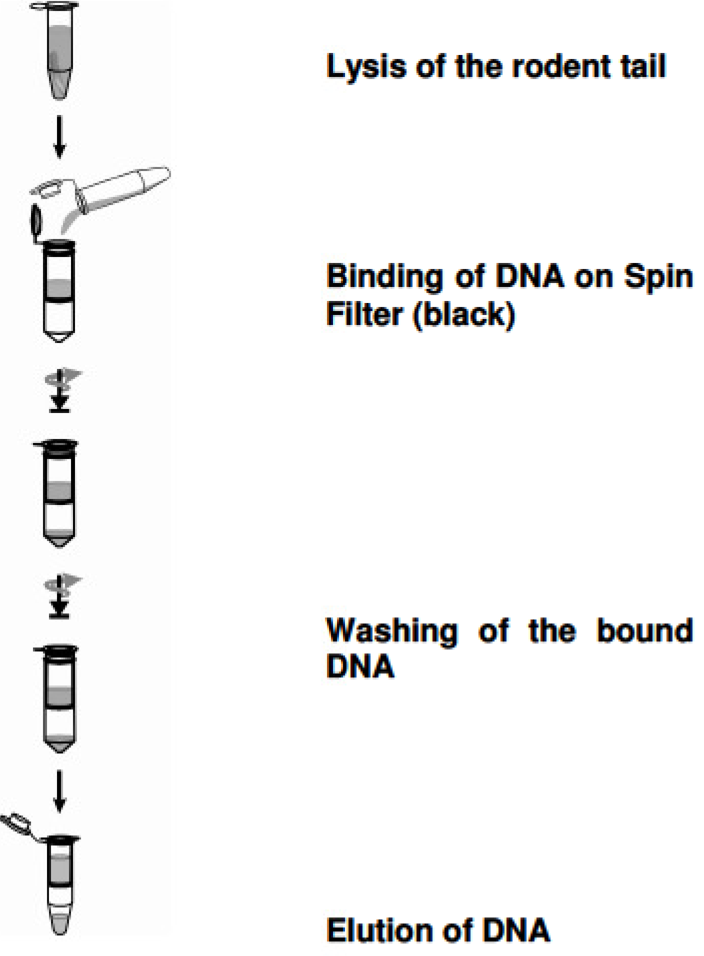
- Pre-heat thermomixer or water bath to 50 °C
- Ensure that the Washing Solution MS and Proteinase K have been prepared according to the instruction (→ "Kit components" p. 5).
- Centrifugation steps should be carried out at room temperature
- Avoid freezing and thawing of starting material

# 8 Components not included in the kit

- RNase A (100 mg/ml); optional
- 1.5 ml tubes
- 2.0 ml tubes; optional
- 96 – 99.8 % ethanol

  **Note:** Use only absolute/pure ethanol, NO methylated or denatured alcohol!
- dd$H_2O$

# 9 General procedure for DNA extraction



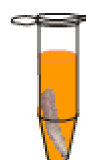
# blackPREP Rodent Tail DNA Kit

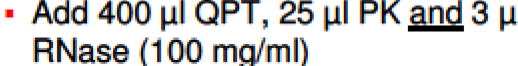
## Protocol: DNA isolation from rodent tails

Recommended steps before starting
- Pre-heat thermomixer or water bath (50 °C)
- Prepare Washing Solution MS and Proteinase K according to the instruction

**1. Starting material**
- Rodent tails
  - Mouse: max. 0.5 – 1.2 cm
  - Rat: max. 0.2 – 0.6 cm

**2. Lysis**





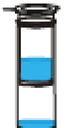
- Add 400 µl QPT, 25 µl PK <u>and</u> 3 µl RNase (100 mg/ml)
- Vortex: 5 sec
- Incubation: 1 – max. 3 h @ 50 °C
- 10.000 x g (~12.000 rpm): 30 sec
- Transfer supernatant; 1.5 ml tube

**3. Binding of DNA**

- Add 200 µl SBS
- Vortex
- Add Spin Filter to Receiver Tube
- Add sample to Spin Filter
- 10.000 x g (~12.000 rpm): 2 min

**4. Washing**

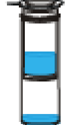
Re-use Receiver Tube



- Add 700 µl MS
- 10.000 x g (~12.000 rpm): 1 min
- Add 700 µl MS
- 10.000 x g (~12.000 rpm): 1 min

**5. Remove Ethanol**

Re-use Receiver Tube



- Discard filtrate
- Add Spin Filter to Receiver Tube
- Centrifuge: max speed, 2 min

**6. Elution**

- Add Spin Filter to an Elution Tube
- Add 200 µl Elution Buffer
- Incubation: 3 min @ RT
- 8.000 x g (~10.000 rpm): 1 min

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

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

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# 10 Product specifications

## 1. Starting material:

- Rodent tails
- Mouse tail max. 0.5 – 1.2 cm
- Rat tail max. 0.2 – 0.6 cm

## 2. Time for isolation:

- Lysis: 1 – max. 3 hours
- Extraction: approx. 9 min

## 3. Typical quality and yield:

- Mouse tail (1.2 cm): 30 – 40 µg
- Rat tail (0.6 cm): 35 – 45 µg
- Ratio $A_{260}$:$A_{280}$: 1.8 – 2.0

## 4. Binding capacity:

- > 100 µg DNA

## 5. Example for isolation of gDNA:

Analysis of extracted gDNA on a 0.8 % TAE agarose gel

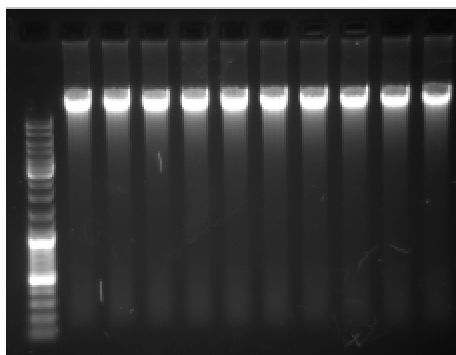

Fig. 1: Isolation of genomic DNA from mouse tail (1.0 cm).

Lane 1: DNA ladder
Lane 2 – 11: Extracted gDNA

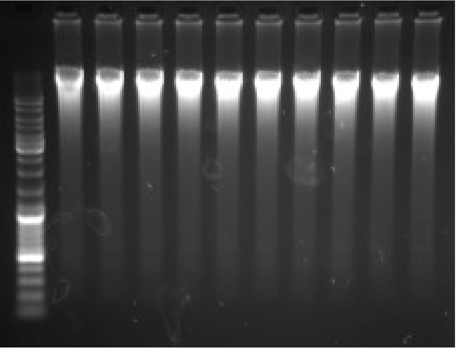

Fig. 2: Isolation of genomic DNA from rat tail (0.5 cm).

Lane 1: DNA ladder
Lane 2 – 11: Extracted gDNA

# 11 Protocol: DNA isolation from rodent tails

1. Place a piece of the rodent tail into a 1.5 ml or 2.0 ml reaction tube (not included in the kit)

   Mouse tail: max. 0.5 – 1.2 cm or

   Rat tail: max. 0.2 – 0.6 cm

2. Add **400 µl Lysis Solution QPT, 25 µl Proteinase K** **and** **3 µl RNase A** (stock solution 100 mg/ml; not included in the kit)
3. Mix vigorously by pulsed vortexing for 5 sec and incubate at 50 °C until the sample is completely lysed (appr. 1 – max. 3 h for rodent tails; check the lysis visually).

   **The lysis step should be finished, if the material is completely or nearly completely lysed!**

   **Note:** We recommend to use a shaking platform (thermomixer, water bath or another rocking platform) for a continuous shaking of the sample. Alternatively, vortex the sample every 10 minutes during the incubation. No shaking will reduce the lysis efficiency.
4. Centrifuge the 1.5 ml reaction tube at 10.000 x g (~12.000 rpm) for 30 sec to spin down unlysed material. Transfer the supernatant into another 1.5 ml reaction tube.
5. Add **200 µl Binding Solution SBS** to the lysed sample, mix by vortexing or by pipetting up and down several times.

   **Note:** It is important that the sample and the Binding Solution SBS are mixed vigorously to get a homogeneous solution.
6. Apply the sample to the Spin Filter (black) 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.
7. Discard the filtrate and place the Spin Filter back into the 2.0 ml Receiver Tube.
8. 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 filtrate and place the Spin Filter back into the 2.0 ml Receiver Tube.
9. Repeat the washing step (point 8) once again

10. Centrifuge at max. speed for 2 min 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 (not included in the kit). Carefully open the cap of the Spin Filter and add **200 µl Elution Buffer**. Incubate at room temperature for 3 minutes. Centrifuge at 8.000 x g (~10.000 rpm) for 1 minute. A second elution step will 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.

# 12 Troubleshooting

| Problem / probable cause | Comments and suggestions |
|---|---|
| **Clogged Spin Filter** | |
| • Insufficient lysis and/or too much starting material | Increase lysis time.<br>Increase centrifugation speed.<br>After lysis centrifuge the lysate to pellet unlysed material.<br>Reduce amount of starting material. |
| **Low amount of extracted DNA** | |
| • Insufficient lysis | Increase lysis time.<br>Reduce amount of starting material.<br>Overloading of Spin Filter reduces yield! |
| • Incomplete elution | Prolong the incubation time with Elution Buffer to 5 min or repeat elution step once again.<br>Take a higher volume of Elution Buffer. |
| • Insufficient mixing with Binding Solution SBS | Mix sample with Binding Solution SBS 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 the DNA with lower volume of Elution Buffer. |
| **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 insufficient | Old material often contains degraded DNA. |
| **RNA contaminations of extracted DNA** | RNase A digestion |

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