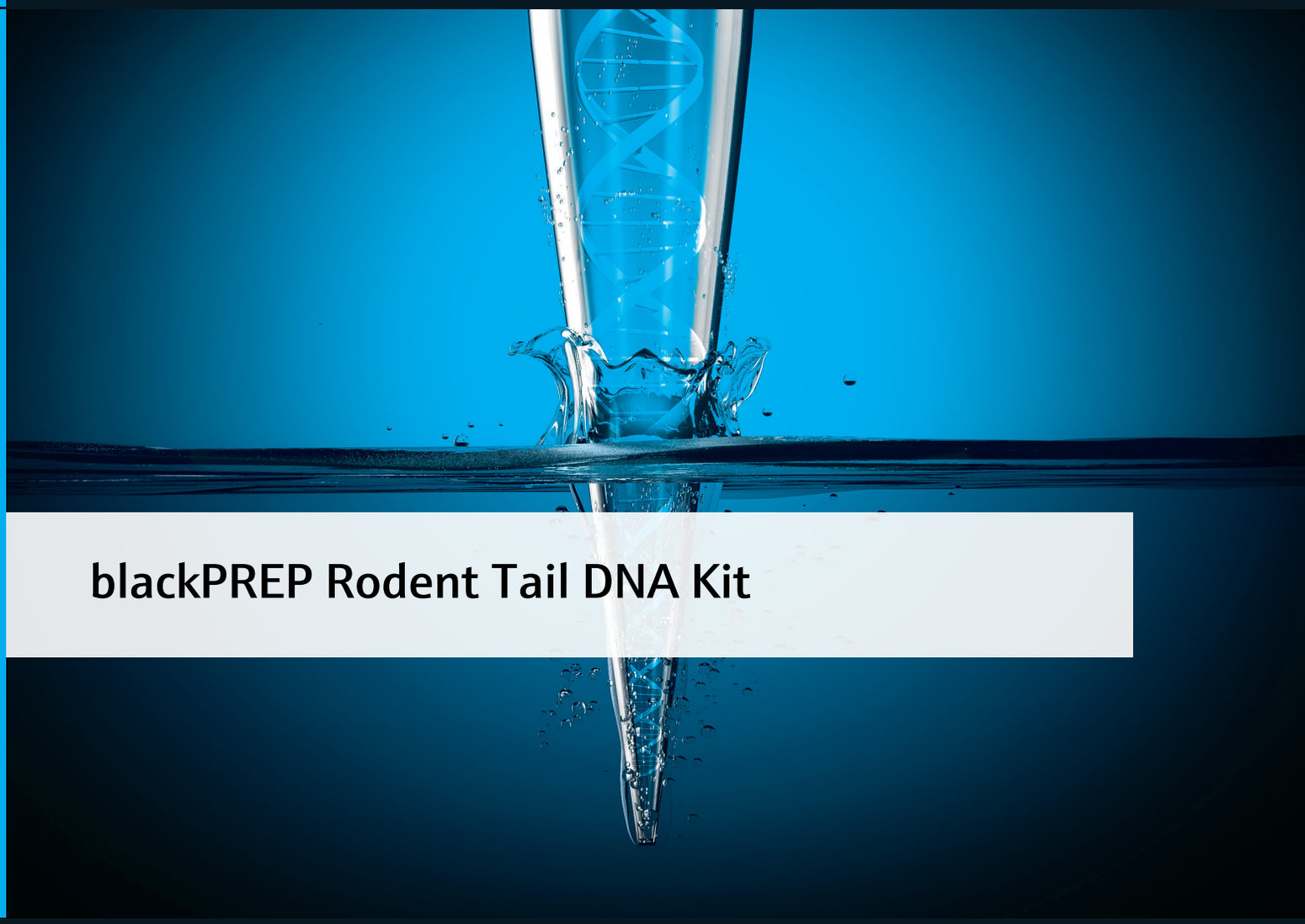
Instructions for Use
Life Science Kits & Assays



blackPREP Rodent Tail DNA Kit

Innuscreen GmbH

Order No.:

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

Publication No.: HB_BP-0010_e_220404

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Made in Germany! info.innu@ist-ag.com

Contents

1 Introduction

1.1 Intended use

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.

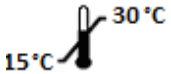
The extraction procedure is based on a new patented technology for isolation of DNA from complex starting materials. The extraction procedure combines a very fast and efficient lysis step with the subsequent binding of genomic DNA on a spin filter surface. The spin filter bounded DNA is washed and the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

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** Catalogue number.
	**Content** Contains sufficient reagents for <N> tests.
	**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 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 and the kit” p. 3).
- Working 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 could 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.

The **blackPREP Rodent Tail 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.

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 blackPREP Rodent Tail Kit or other IST Innuscreen GmbH products, please do not hesitate to contact us.

For technical support or further information in Germany 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 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 Components included in the kit

	Σ 10	Σ 50	Σ 250
REF	845-BP-0010010	845-BP-0010050	845-BP-0010250
Lysis Solution QPT	5 ml	25 ml	120 ml
Binding Solution SBS	5 ml	15 ml	60 ml
Proteinase K	for 1 x 0.3 ml working solution	for 1 x 1.5 ml working solution	for 4 x 1.5 ml working solution
Washing Solution MS (conc.)	6 ml	24 ml	2 x 60 ml
Elution Buffer	2 x 2 ml	15 ml	60 ml
Spin Filter	10	50	5 x 50
Receiver Tubes	50	5 x 50	25 x 50
Elution Tubes	10	50	5 x 50
Manual	1	1	1

6.2 Components not included in the kit

- RNase A (100 mg/ml); optional
- 1.5 ml reaction tubes
- 2.0 ml reaction tubes
- 96 – 99.8 % ethanol (non-methylated or denatured)
- ddH_2O

7 Product specifications

1. Starting material

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

2. Time for extraction

- 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

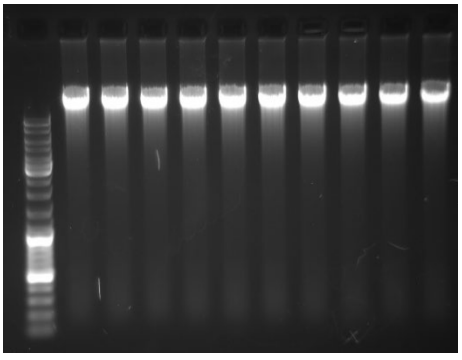


Fig. 1: Isolation of genomic DNA from mouse tail (1.0 cm).

Lane 1: DNA ladder

Lane 2 – 11: Extracted gDNA

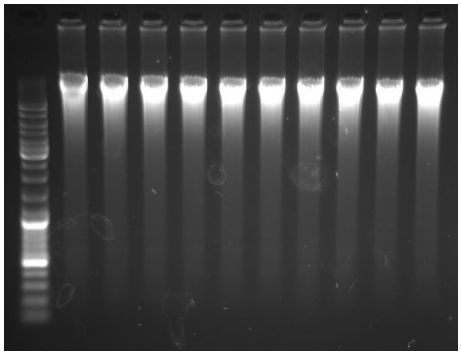


Fig. 2: Isolation of genomic DNA from rat tail (0.5 cm).

Lane 1: DNA ladder

Lane 2 – 11: Extracted gDNA

8 Initial steps before starting

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

845-BP-0010010	Add 14 ml ethanol to 6 ml Washing Solution MS (conc.).
845-BP-0010050	Add 56 ml ethanol to 24 ml Washing Solution MS (conc.).
845-BP-0010250	Add 140 ml ethanol to 60 ml Washing Solution MS (conc.).

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

845-BP-0010010	Add 0.3 ml ddH2O to lyophilized Proteinase K.
845-BP-0010050	Add 1.5 ml ddH2O to lyophilized Proteinase K.
845-BP-0010250	Add 1.5 ml ddH2O to lyophilized Proteinase K.

9 Protocol for DNA isolation from rodent tails

1. 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 (approx. 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 using 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 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 – 8 °C. For long time storage placing at –22 °C.to – 18 °C is recommended.

10 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.
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 min or repeat elution step once again. 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 concentrate 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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