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Manual

blackPREP Swab DNA Kit

Life Science unlimited

Order No.:

845-BP-0030010	10 reactions
845-BP-0030050	50 reactions
845-BP-0030250	250 reactions

Publication No.: HB_BP-0030_e_120116

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Contents

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 blackPREP Swab 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 blackPREP Swab DNA Kit were tested by isolation of genomic DNA from tissue sample 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 blackPREP Swab 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**
Buccal Swabs	10	50	250
Lysis Solution TLS	5 ml	25 ml	120 ml
Binding Solution TBS	5 ml	25 ml	120 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 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	15 ml	60 ml
Pre-filter (clear)	10	50	5 x 50
Spin Filter (black)	10	50	5 x 50
Receiver Tubes (2.0 ml)	50	5 x 50	25 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 to 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

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

8 General procedure for DNA extraction

Transfer of the swab to a reaction tube

Lysis of starting material

Binding of DNA on Spin Filter (blue)

Washing of the bound DNA

Elution of DNA

9 Product specifications

1. Starting material:

Buccal swabs

2. Time for isolation:

Approximately 20 – 25 minutes

3. Typical quality and yield:

- Depends on type and amount of starting material
- Binding capacity of the spin column is > 100 µg DNA
- Up to 20 µg DNA
- Ratio A_{260}:A_{280}: 1.7 – 2.0

4. Example for isolation of DNA:

Analysis of extracted DNA on a 1.0 % TBE agarose gel

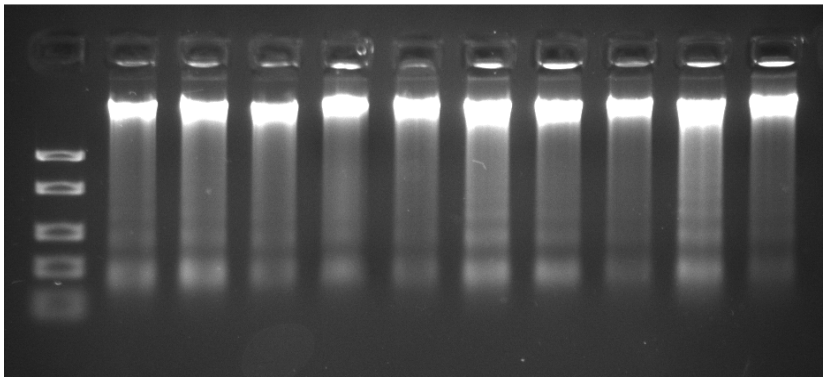

Fig. 1: Isolation of DNA from different buccal swab samples
Lane 1: DNA ladder
Lane 2 – 11: Extracted DNA

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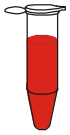
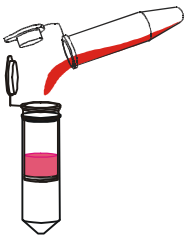
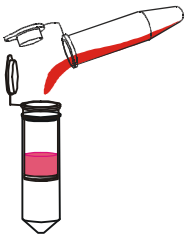
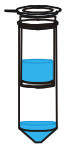
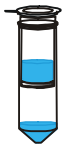
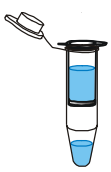
Protocol: DNA isolation from buccal swab

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	▪ Buccal Swab	▪ Place the swab into a 1.5 ml tube
2.	Lysis		▪ Add 400 µl TLS and 25 µl PK ▪ Vortex: 5 sec ▪ Incubation: 50 °C; 10 – 15 min
3.	Pre-filtration	 	▪ Add Pre-filter to Receiver Tube ▪ Add sample (swab and liquid) to Pre-filter ▪ 10.000 x g (~12.000 rpm): 1 min
4.	Binding of DNA	 	▪ Add 400 µl TBS ▪ Vortex: 15 sec ▪ Add Spin Filter to Receiver Tube ▪ Add sample to Spin Filter ▪ 10.000 x g (~12.000 rpm): 2 min
5.	Washing New Receiver Tube	 	▪ Add 500 µl HS ▪ 10.000 x g (~12.000 rpm): 1 min ▪ Add 750 µl MS ▪ 10.000 x g (~12.000 rpm): 1 min
6.	Remove Ethanol New Receiver Tube	 	▪ Discard filtrate ▪ Add Spin Filter to Receiver Tube ▪ Centrifuge: max speed, 2 min
7.	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

✂ 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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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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10 Protocol: DNA isolation from Buccal Swab

Important

To get maximum yield of DNA it is essential to leave the swab during the complete lysis time in the 1.5 ml tube. It is possible to cut the shaft of the swab, so that you can close the cap of the tube. The removal of the swab from the tube ahead of time will lead to a dramatically reduced final yield!

1. Place the **Buccal Swab** into a 1.5 ml reaction tube and snap the shaft (the head of the swab has to fit inside the 1.5 ml reaction tube completely).
2. Add **400 µl Lysis Solution TLS and 25 µl Proteinase K**, mix vigorously by pulsed vortexing for 5 sec and incubate at 50 °C for 10 – 15 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.

   After lysis time transfer the snapped Buccal Swab together with the liquid (lysed) sample from the 1.5 ml reaction tube onto a Prefilter (mauve) located in a 2.0 ml Receiver Tube. Close the cap of the Prefilter.
3. Centrifuge for 1 minute at 10.000 x g (~12.000 rpm). Remove and discard the Prefilter
4. Add **400 µl Binding Solution TBS** to the filtrate, mix by vortexing or by pipetting up and down several times.

Please be careful!

Mechanical stress by vortexing or extensive mixing leads to shearing of high-molecular weight chromosomal DNA. But it is important that the sample and the Binding Solution TBS are mixed completely to get a homogeneous solution.

5. Apply the sample onto a 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.

6. 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.
7. Open the Spin Filter and add **750 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.
8. Centrifuge at max. speed for 2 minutes to remove all traces of ethanol. Discard the 2.0 ml Receiver Tube.
9. 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. 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.

11 Troubleshooting

Problem / probable cause	Comments and suggestions
Low amount of extracted DNA	
• Insufficient lysis	Increase lysis time. Leave the swab inside the 1.5 ml reaction tube with Lysis Solution TLS during the complete lysis time.
• 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 TBS	Mix sample with Binding Solution TBS by pipetting or by vortexing prior to transfer of the sample onto the Spin Filter.
Low concentration of extracted DNA	
• Loss of lysate	Transfer the whole liquid and the buccal swab (snapped) onto the Prefilter unit, after the lysis step.
• Too much Elution Buffer	Elute the DNA with lower volume of Elution Buffer.
Degraded or sheared DNA	
• Old material	Old blood contains degraded DNA or apoptotic DNA.
RNA contaminations of extracted DNA	RNase A digestion

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