Instructions for Use
Life Science Kits & Assays

blackPREP FFPE DNA Kit

analytikjena
An Endress+Hauser Company

Order No.:
845-BP-0021010 10 reactions
845-BP-0021050 50 reactions
845-BP-0021250 250 reactions

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1 Introduction

1.1 Intended use

The kit has been designed as a very efficient tool for fast isolation of genomic DNA from FFPE samples. The kit can be used with different amount of FFPE tissue samples / sections, up to a maximum amount of tissue of 50 mg.
For detailed information see Product specifications (→ "Product specifications", p. 12).

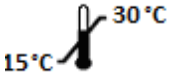
The extraction procedure is based on a new patented chemistry and combines lysis of FFPE tissue samples with subsequent binding of nucleic acids onto the surface of a Spin Filter membrane. After several washing steps the nucleic acids are eluted from the membrane by using Elution Buffer. The extraction chemistry and protocol are optimized to get maximum of yield.

The blackPREP FFPE DNA Kit is not designed for use with other starting materials as described above and the kit performance has not been evaluated for other starting materials.

The kit is intended for use by professional users. The kit has been designed to be used for a wide range of different downstream applications, like amplification reactions.

1.2 Notes on the use of this manual

For easy reference and orientation, the manual uses the following warning and information symbols as well as the shown methodology:

Symbol	Information
	**REF** Catalogue number.
 N	**Content** Contains sufficient reagents for <N> reactions.
	**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.

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“ p. 3).
- Working steps are numbered.

2 Safety precautions

NOTE

Read through this chapter carefully prior 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, please ask for the Safety Data Sheet (SDS).

3 Storage conditions

Store lyophilized **Proteinase K** at 4 °C to 8 °C! Divide dissolved **Proteinase K** into aliquots and store at -22 °C to -18 °C. Repeated freezing and thawing will reduce the activity dramatically!

All other components of the blackPREP FFPE 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.

Before every use make sure that all components have room temperature. If there are any precipitates within the provided solutions dissolve these precipitates by careful warming.

For further information see chapter ”Kit components“ p.7.

4 Functional testing and technical assistance

The Analytik Jena AG 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 FFPE 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 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. 9). Since the performance characteristics of Analytik Jena AG kits have just been validated for the application described above, the user is responsible for the validation of the performance of Analytik Jena AG kits using other protocols than those described below. 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 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
The kit is for research use only!

6 Kit components

IMPORTANT
Store lyophilized **Proteinase K** at 4 °C to 8 °C! Aliquot dissolved **Proteinase K** and store at -22 °C to -18 °C. Repeated freezing and thawing will reduce the activity dramatically!

STORAGE CONDITIONS
All other components are stored at room temperature.

	Σ 10	Σ 50	Σ 250
REF	845-BP-0021010	845-BP-0021050	845-BP-0021250
Lysis Solution MA	5 ml	25 ml	120 ml
Proteinase K	for 2 x 0.3 ml working solution	for 2 x 1.5 ml working solution	for 7 x 1.5 ml working solution
Washing Solution C	8 ml	30 ml	135 ml
Washing Solution BS (conc.)	2 ml	10 ml	18 ml
Elution Buffer	2 x 2 ml	15 ml	2 x 30 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

	Σ 10	Σ 50	Σ 250
Initial steps	**Proteinase K** Dissolve Proteinase K by addition of 0.3 ml ddH_2O, mix thoroughly and store as described above! **Washing Solution BS** Add 18 ml of 96-99.8 % ethanol to the bottle and mix thoroughly. Keep the bottle always firmly closed!	**Proteinase K** Dissolve Proteinase K by addition of 1.5 ml ddH_2O, mix thoroughly and store as described above! **Washing Solution BS** Add 90 ml of 96-99.8 % ethanol to the bottle and mix thoroughly. Keep the bottle always firmly closed!	**Proteinase K** Dissolve Proteinase K by addition of 1.5 ml ddH_2O, mix thoroughly and store as described above! **Washing Solution BS** Add 162 ml of 96-99.8 % ethanol to the bottle and mix thoroughly. Keep the bottle always firmly closed!

COMPONENTS NOT INCLUDED IN THE KIT

- 1.5 ml reaction tubes
- 2.0 ml reaction tubes
- ddH_2O for dissolving **Proteinase K**
- 96–99.8 % ethanol, non-denatured or methylated
- RNase A (10 mg/ml); optional

7 Product specifications

1. **Starting material:**
   - FFPE (formalin fixed paraffin embedded) tissue samples
   - Approx. 8 mg (approx. 12 µl) paraffin correspond to:
     ≈ 4 sections of 10 µm thickness and each of 300 mm^2 area
     ≈ 3 sections of 10 µm thickness and each of 400 mm^2 area
     ≈ 2 sections of 10 µm thickness and each of 600 mm^2 area
     ≈ 1 sections of 10 µm thickness and each of 1,200 mm^2 area
   - Maximum amount of tissue: 50 mg

NOTE
It is possible to process more than the amount of starting material indicated above. In such case, it is the costumer's responsibility to validate the blackPREP FFPE DNA Kit for this new purpose.

2. **Time for isolation:**
   - Approximately 2.5 hours (all steps included)

3. **Typical yield:**
   - Depends on type and amount of starting material
   - The extracted genomic DNA (gDNA) can be used for a wide range of different molecular biology applications.

8 GHS classification

Component	Hazard contents	GHS Symbol	Hazard phrases	Precaution phrases
Washing Solution C (conc.)	Propan-2-ol 25–50 %	 Danger	225, 319, 336	101, 102, 103, 210, 261,303+361+353, 305+351+338, 405, 501
Proteinase K	Proteinase, engyodontium album 50-100 %	 Danger	315, 319, 334, 317, 335	101, 102, 103, 261, 280, 305+351+338, 342+311, 405, 501

8.1 Hazard phrases

225	Highly flammable liquid and vapour.
315	Causes skin irritation.
317	May cause an allergic skin reaction.
319	Causes serious eye irritation.
334	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
335	May cause respiratory irritation.
336	May cause drowsiness or dizziness.

8.2 Precaution phrases

101	If medical advice is needed, have product container or label at hand.
102	Keep out of reach of children.
103	Read label before use.
210	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
261	Avoid breathing dust/fume/gas/mist/vapors/spray.
280	Wear protective gloves/protective clothing/ eye protection/face protection.
405	Store locked up.
501	Dispose of contents/container in accordance with local/regional/national/international regulations.
342+311	If experiencing respiratory symptoms: Call a POISON CENTER/doctor.
303+361+353	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.
305+351+338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

9 Recommended steps before starting

- Ensure that the **Proteinase K and Washing Solution BS** have been prepared according to the instruction (→ “Kit components” p. 7).
- Heat thermal mixer or water bath to 65 °C, followed by 90 °C.
- Centrifugation steps should be carried out at room temperature.
- Avoid freezing and thawing of starting material.

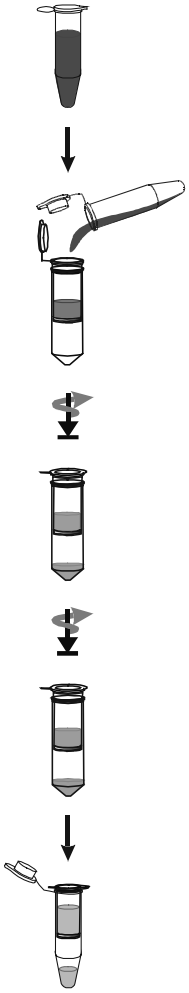
10 Extraction procedure

10.1 Summary

The kit has been designed as a tool for very fast and efficient isolation of genomic DNA from FFPE samples. The extraction procedure is based on a new kind of chemistry, which combines an efficient lysis step with a subsequent efficient binding of genomic DNA on a Spin Filter surface followed by washing of the bound DNA and finally eluting of the DNA. The recovery of DNA and the quality are excellent. The new kind of chemistry allows the isolation of DNA from FFPE samples without the deparaffinising step using toxic and hazardous components like octane or xylene.

The extraction process is finished within 2.5 hours. The isolated DNA is suitable for amplification reaction and other amplification based further downstream applications.

10.2 General extraction principle



Lysis of starting material

Binding of DNA on Spin Filter

Washing of bound DNA

Elution of DNA

11 Examples of application

11.1 Extraction of different FFPE tissue sample

- Extraction of 12 different FFPE tissue samples (approx. 3 x 7 µm each sample)
- Spectrophotometric measurement of all 12 samples
- Subsequent amplification of a human specific target sequence

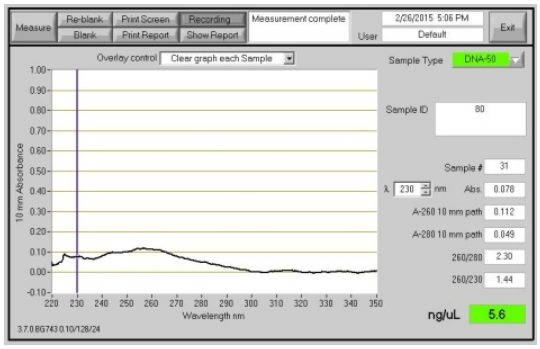
Results

After the extraction process, the amount of isolated DNA (eluted in 100 µl Elution Buffer) was measured by spectrophotometric method and subsequent amplification of a human specific target sequence by Real-time PCR:

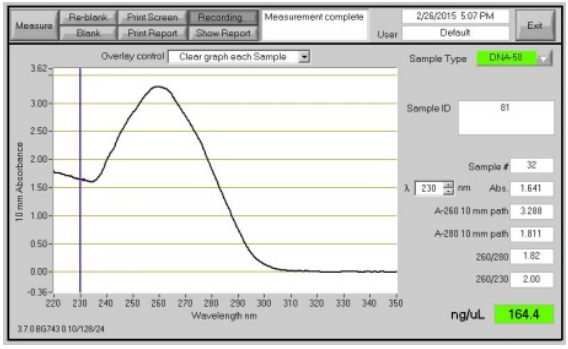
Sample ID	DNA [ng/µl]	Ratio A260/280	Ratio A260/230	Ct values	
1	5.78	1.95	1.50	30.88	31.22
2	159.27	1.82	1.96	24.41	23.55
3	50.85	1.70	1.59	27.40	27.51
4	60.80	1.83	2.18	26.85	26.85
5	34.09	1.85	2.23	29.48	29.47
6	14.93	2.03	2.35	27.94	27.91
7	195.22	1.81	2.18	27.43	27.27
8	122.79	1.84	2.28	25.61	25.60
9	56.45	1.80	2.19	28.49	28.72

10	109.9	1.66	1.79	30.21	30.42
11	50.18	1.78	2.22	27.74	28.99
12	15.54	1.85	2.01		

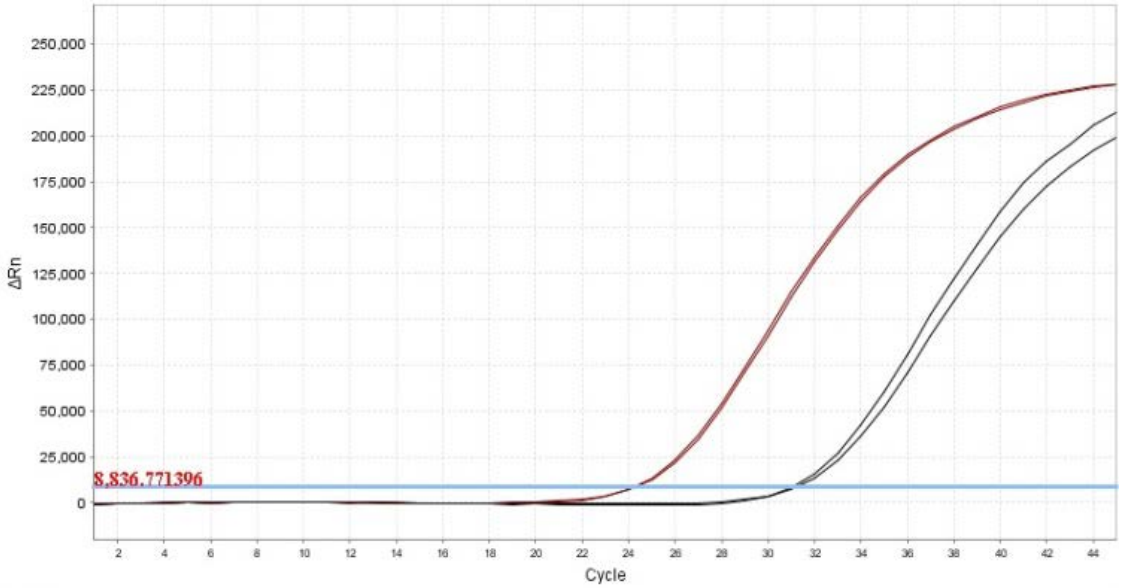
Analysis of sample 1 (adipose tissue) and sample 2 (pancreatic tissue):



Sample 1: FFPE tissue sample with low yield.

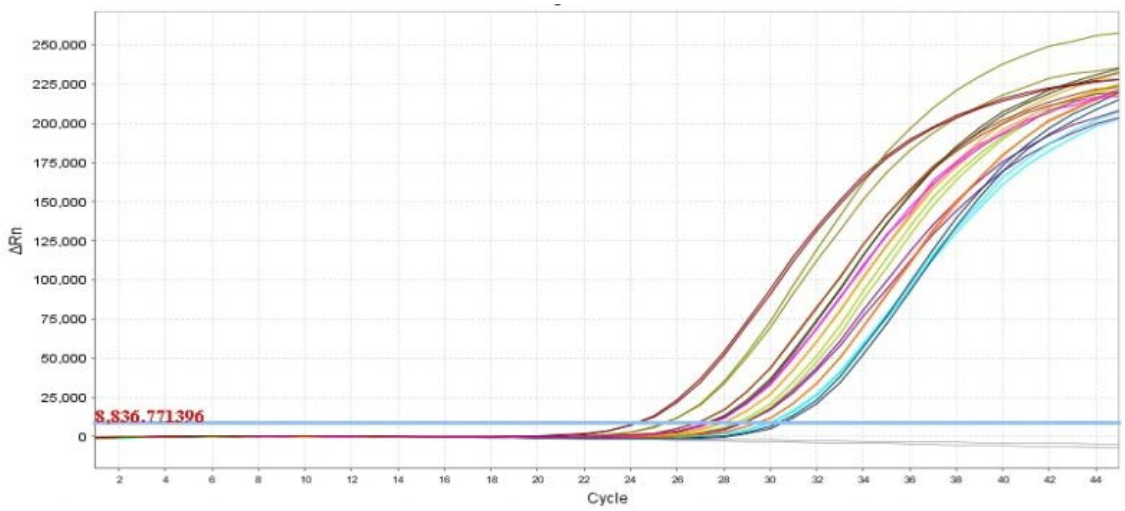


Sample 2: FFPE tissue sample with high yield.



The Ct values of sample 1 (■) and sample 2 (■) correspond to the yield as shown above.

Analysis of sample 3 up to sample 11 (pancreatic tissue) and sample 12 (duodenum tissue):



The Ct values of different FFPE tissue samples correspond to the yield as shown above. Sample 3 (■), sample 4 (■), sample 5 (■), sample 6 (■), sample 7 (■), sample 8 (■), sample 9 (■), sample 10 (■), sample 11 (■) and sample 12 (■).

Data kindly provided by Dr. L.F.Grochola, Department of Research / Centre for Surgery, University Hospital Zurich, Switzerland.

11.2 DNA Extraction trom FFPE samples with different amount of starting material

- DNA Extraction from FFPE tissue samples with different amount of starting material
- Spectrophotometric measurement of all samples
- Subsequent amplification of a human specific target sequence

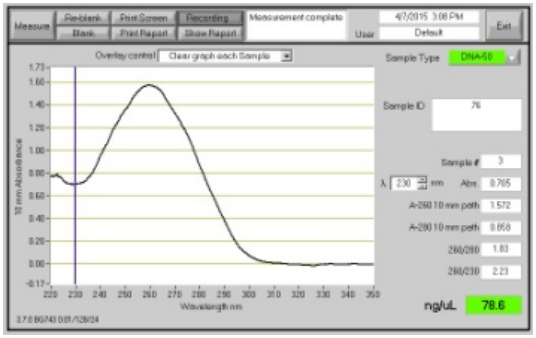
Sample ID	No. of sections	Section thickness	Weight of tissue	Paraffin surface
A	4	10 µm	~ 2 mg	1,200 mm²
B	2	10 µm	~ 2 mg	600 mm²
C	1	10 µm	~ 2 mg	300 mm²
D	2	10 µm	~ 4 mg	600 mm²
E	3	10 µm	~ 6 mg	900 mm²

Results

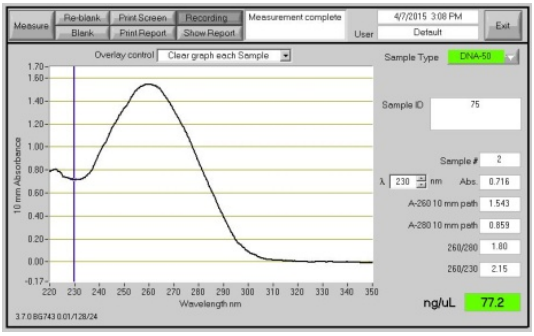
After the extraction process, the amount of isolated DNA (eluted in 100 µl Elution Buffer) was measured by spectrophotometric method and subsequent amplification of a human specific target sequence by Real-time PCR:

Sample ID	DNA [ng/µl]	Ratio A260/280	Ratio A260/230	Ct values
A	78.6	1.83	2.23	29.67 29.58
B	77.2	1.80	2.15	29.63 29.96
C	74.6	1.73	2.12	29.71 29.76
D	116.8	1.83	2.21	28.91 28.75
E	261.5	1.79	2.14	28.28 28.33

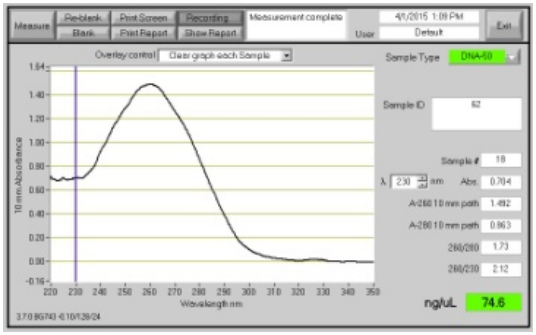
Spectrophotometric analysis of sample A–E:



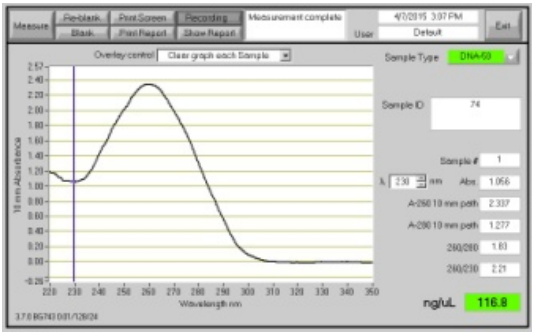
Sample A: 4 sections, 2 mg tissue, 1,200 mm^2 paraffin.



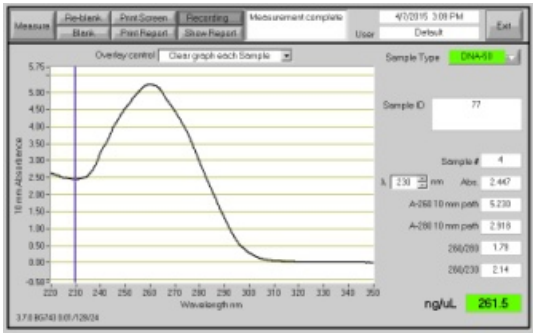
Sample B: 2 sections, 2 mg tissue, 600 mm^2 paraffin.



Sample C: 1 section, 2 mg tissue, 300 mm^2 paraffin.

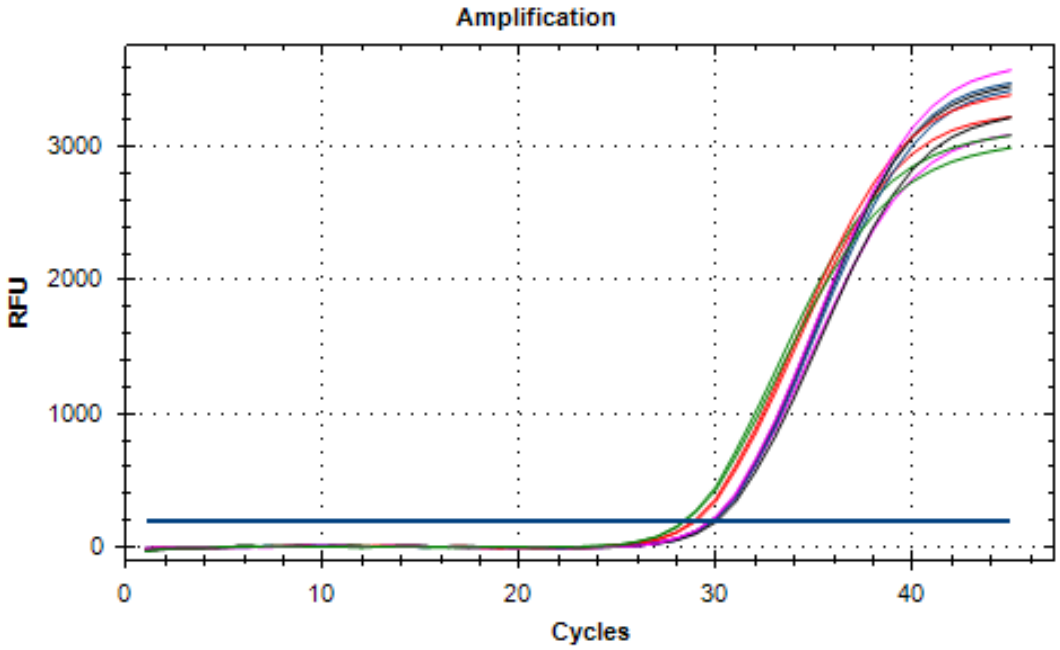


Sample D: 2 sections, 4 mg tissue, 600 mm^2 paraffin.



Sample E: 3 sections, 6 mg tissue, 900 mm^2 paraffin.

Analysis of sample A up to sample E by Real-time PCR:



The Ct values of different FFPE tissue samples correspond to the yield as shown above. Sample A (■), sample B (■), sample C (■), sample D (■) and sample E (■).

12 Protocol: DNA isolation from paraffin embedded tissue samples

1. Place the **FFPE material** into a 1.5 ml or 2.0 ml reaction tube and centrifuge the reaction tube at maximum speed for 1 minute.

NOTE

For correct sample amount see „Product specifications“ on p. 9.

2. Open the reaction tube and add **400 µl Lysis Solution MA and 40 µl Proteinase K** to the sample, mix vigorously by pulsed vortexing for 10 seconds.

IMPORTANT

The FFPE material has to be completely covered by the **Lysis Solution MA**, if necessary spin down briefly to remove drops from the lid!

3. Incubate the reaction tube at 65 °C for 1 hour in a thermal mixer under continuous shaking at 1,000 rpm.

NOTE

We recommend using 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.

IMPORTANT

If the residual tissue sample is still visible after 1 hour, prolong the incubation step until the tissue is completely lysed.

4. After lysis step place the sample into a thermal mixer pre-heated to 90 °C and incubate the sample for 1 hour under continuous shaking at 1,000 rpm.

IMPORTANT

Do not place the sample into the thermal mixer, before the temperature of 90 °C is achieved! Longer incubation at 90 °C may lead to lower yield!

5. Incubate the sample for 5 minutes at room temperature.

NOTE

To remove RNA from the sample (if necessary) add 10 µl of RNase A solution (10 mg/ml), vortex shortly and incubate for 5 min at room temperature. RNA removal from the sample by RNase A could lead partial loss of DNA.

6. Centrifuge the sample at maximum speed for 2 minutes and transfer the sample as much as possible into a new 1.5 ml reaction tube under considering following notes. Try to avoid carryover of residual FFPE material!

NOTE

Depending on the amount of paraffin material in the sample, a layer forms on the top of lysed sample. Try to avoid carryover of this formed paraffin layer in a new 1.5 ml reaction tube.

Depending on the sample a pellet forms on the bottom of the reaction tube. Avoid to carryover this pellet in a new 1.5 ml reaction tube.

7. Add **400 µl of ethanol absolute (96–99 %)** to the sample, mix vigorously by pulsed vortexing for 10 seconds or pipetting up and down several times.

NOTE

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

8. Apply the sample onto a Spin Filter located in a Receiver Tube. Close the cap and centrifuge at 10,000 x g (~12,000 rpm) for 1 minute.

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 Receiver Tube.

9. Open the Spin Filter and add **500 µl Washing Solution C**, 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 Receiver Tube.

10. Open the Spin Filter and add **650 µl Washing Solution BS**, 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 Receiver Tube.

11. Open the Spin Filter and add **650 µl ethanol absolute (96–99 %)**, 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 Receiver Tube.

12. Centrifuge at maximum speed for 3 minutes to remove all traces of ethanol. Discard the Receiver Tube.
13. Place the Spin Filter into an 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 10,000 x g (~12,000 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 to 8 °C. For long time storage placing at -18 °C to -22 °C is recommended.

13 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.
Paraffin layer on lysed sample	
Too much starting material	If the lysed sample is covered by a paraffin layer after centrifugation step (step 6). Transfer carefully all solution by piercing the paraffin layer using a 100 µl pipette. Avoid carryover of paraffin to the next tube!
Low amount of extracted DNA	
Insufficient lysis	Increase lysis time! Reduce amount of starting material. Overloading 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 ethanol absolute	Mix sample with ethanol absolute by pipetting or by vortexing prior to transfer of the sample onto the Spin Filter.
Low concentration of extracted DNA	
Too much Elution Buffer was used in the elution step	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 after taking in liquid nitrogen or at -18 °C to -22° C! For long time storage continuously store at -78 °C to -82° C! Avoid thawing of the material.
Old starting material	Old material often contains degraded DNA. Repeat with fresh material.

Problem / probable cause	Comments and suggestions
RNA contamination	
Extracted DNA is contaminated with RNA	Perform an RNase A digestion.
Insufficient quality of extracted DNA	
Carryover of paraffin or pellet	Carefully transfer all solution by piercing the paraffin layer using a 100 µl pipette after centrifugation step (step 6). Avoid carryover of paraffin to the next tube!
Sample diffuses out from the electrophoresis gel	
Ethanol was not completely removed from the Spin Filter	Centrifuge at maximum speed for 3 minuntes (step 12) with open lid of the Spin Filter. Prolong the centrifugation step at maximum speed before elution step (step 12). Incubate eluted samples in elution tube with opened lied at 37 °C for 30 minutes.

14 Related products

Name	Amount	Order No.
Products for PCR & Gel Electrophoresis		
innuPREP DOUBLEpure Kit	10 rxn	845-KS-5050010
	50 rxn	845-KS-5050050
	250 rxn	845-KS-5050250
innuPREP Gel Extraction Kit	10 rxn	845-KS-5030010
	50 rxn	845-KS-5030050
	250 rxn	845-KS-5030250
innuPREP PCRpure Kit	10 rxn	845-KS-5010010
	50 rxn	845-KS-5010050
	250 rxn	845-KS-5010250
innuTaq DNA Polymerase (5 U/µl)	500 U	845-EZ-1000500
50x inNucleotide Mix (1.5 mM)	2 x 0.5 ml	845-AS-9000100
inNucleotide Set (100 mM)	4 x 0.25 ml	845-AS-1100250
innuMIX rapidPCR MasterMix	100 rxn	845-AS-1600100
	200 rxn	845-AS-1600200
innuMIX Standard PCR MasterMix	100 rxn	845-AS-1700100
	200 rxn	845-AS-1700200
innuMIX Green PCR MasterMix	100 rxn	845-AS-1400100
	200 rxn	845-AS-1400200
innuSTAR 100 bp DNA Ladder Express	500 µl	845-ST-1010100
	5 x 500 µl	845-ST-1010500
innuSTAR 1 kb DNA Ladder Express	500 µl	845-ST-1020100
	5 x 500 µl	845-ST-1020500
6x Loading Dye Bromophenol Blue	3 x 1.0 ml	845-ST-3010003
	6 x 1.0 ml	845-ST-3010006

engl. 08/18 – Analytik Jena AG, Jena

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Pictures: Analytik Jena AG
Subject to changes in design and scope of delivery as well as further technical development!