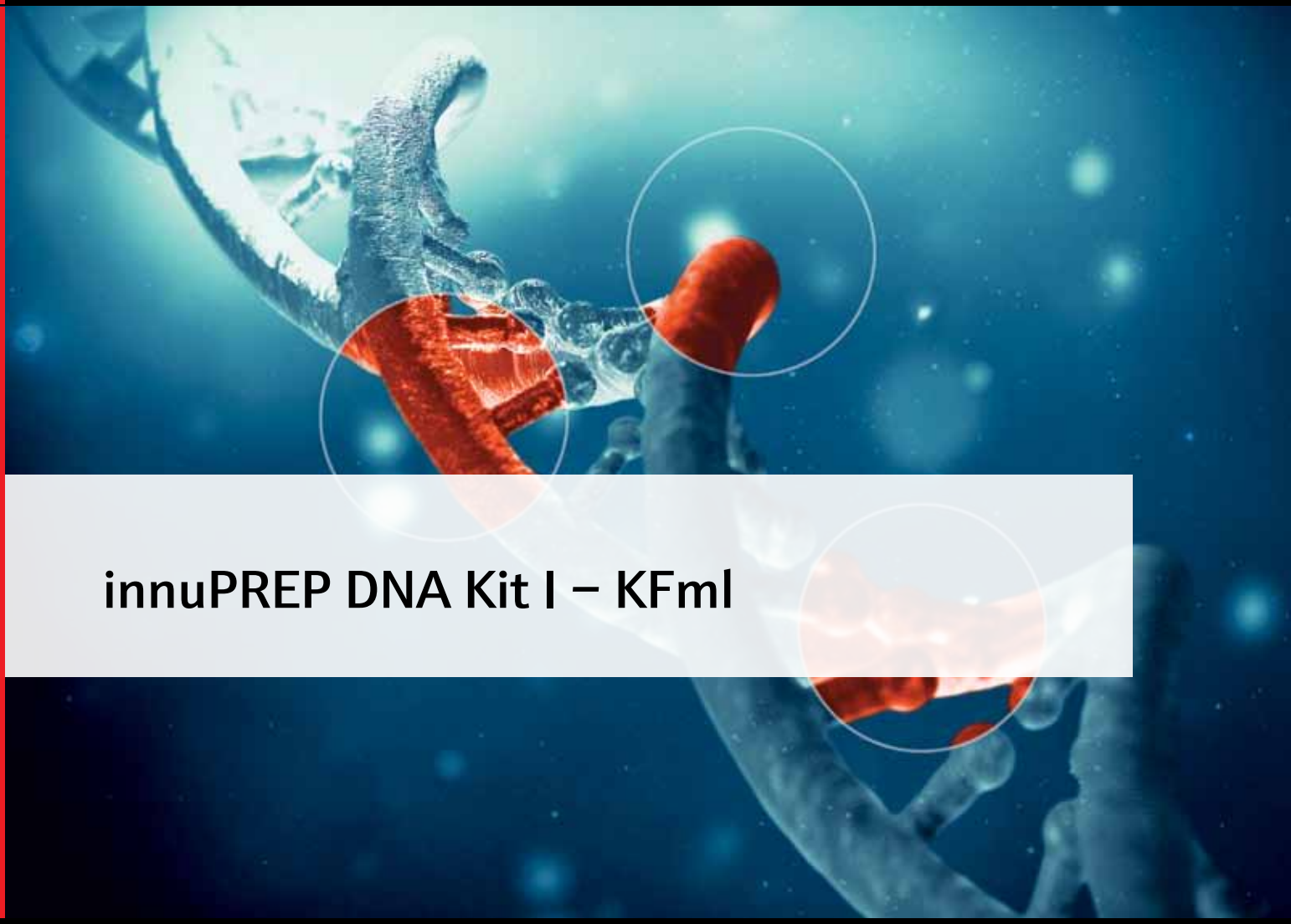
Instructions for Use
Life Science Kits & Assays



innuPREP DNA Kit I – KFml

analytikjena
An Endress+Hauser Company

Order No.:
845-KF-8015015 15 reactions
845-KF-8015250 250 reactions

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Manufacturer:
AJ Innuscreen GmbH
Robert-Rössle-Straße 10
13125 Berlin
Made in Germany!

Distribution/Publisher:
Analytik Jena AG
Konrad-Zuse-Straße 1
07745 Jena · Germany

Phone +49 3641 77 9400
Fax +49 3641 77 767776
www.analytik-jena.com
info@analytik-jena.com

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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 innuPREP DNA Kit I – KFml 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 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.

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 innuPREP DNA Kit I – KFml 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 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 to 8 °C! Divide dissolved **Proteinase K** into aliquots and storage at -22 °C to -18 °C is recommended. Repeated freezing and thawing will reduce the activity dramatically! Store the **MAG Suspension** at 4 °C to 8 °C. All other components are stored at room temperature.

	15 extractions	**250 extractions**
Lysis Solution BLB	5 ml	80 ml
MAG Suspension	1 ml	3 x 5.5 ml
Lysis Solution QPT	8 ml	125 ml
Proteinase K	for 0.3 ml working solution	for 4 x 1.5 ml working solution
Washing Solution HS (conc.)	5 ml	70 ml
Washing Solution MS (conc.)	9 ml	2 x 66 ml
Elution Buffer	2 x 2 ml	60 ml
KFml Tip Combs	3	50
KFml Tube Strips	15	250
Manual	1	1
Initial steps	• Add 5 ml of 96-99.8 % ethanol to the bottle Washing Solution HS, mix thoroughly and keep the bottle always firmly closed! • Add 21 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 70 ml of 96-99.8 % ethanol to the bottle Washing Solution HS, mix thoroughly and keep the bottle always firmly closed! • Add 154 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!

6 GHS classification

Component	Hazard contents	GHS Symbol	Hazard phrases	Precaution phrases	EUH
Lysis Solution BLB	Sodium N-lauroylsarcosinate 2.5-<10 % Guanidinium chloride 0.1-<2.5 %	 *Warning*	319	101, 102, 103, 261, 280, 264, 305+351+338, 310, 304+340	
Washing Solution HS	Guanidinium thiocyanate 50–100 %	 *Danger*	302, 314, 412	101, 102, 103, 260,303+361+353, 305+351+338, 310, 405, 501	032
Proteinase K	Proteinase, Tritirachium album serine	 *Danger*	315, 319, 334, 317, 335	101, 102, 103, 261, 280, 305+351+338, 342+311, 405, 501	

Caution: DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

Hazard phrases

302 Harmful if swallowed.

314 Causes severe skin burns and eye damage.

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.

412 Harmful to aquatic life with long lasting effects.

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.

260 Do not breathe dust/fume/gas/mist/vapors/spray.

261 Avoid breathing dust/fume/gas/mist/vapors/spray.

264 Wash thoroughly after handling.

280 Wear protective gloves/protective clothing/ eye protection/face protection.

310 Immediately call a POISON CENTER/doctor.

405 Store locked up.

501 Dispose of contents/container in accordance with local/regional/national/international regulations.

304+340 IF INHALED: Remove person to fresh air and keep comfortable for breathing.

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.

EU hazard statements

032 Contact with acids liberates very toxic gas

7 Recommended steps before starting

- Pre-heat a thermal mixer or water bath to 50°C, 70 °C or 90 °C (depending on protocol)
- 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

8 Components not included in the kit

- 1.5 ml and 2.0 ml reaction tubes
- 96 – 99.8 % ethanol

  Note: Use only absolute/pure ethanol, NO methylated or denatured alcohol!
- ddH_2O
- Isopropanol (molecular biology grade)
- RNase A (stock solution 10 mg/ml)

9 Protocol 1: Isolation of DNA from whole blood

A. Lysis of sample

1. Transfer **150 µl** of the **whole blood sample** into a 1.5 ml reaction tube.
2. Add **250 µl Lysis Solution BLB and 20 µl Proteinase K**. Vortex the 1.5 ml reaction tube for 10 seconds.
3. Incubate the 1.5 ml reaction tube at 70°C for 20 minutes while shaking continuously (thermal mixer).

B. Preliminary steps of the KingFisher ml

1. During sample lysis pre-fill the tubes of the KingFisher Tube Strips with the following buffers respectively.

Tube A: 400 µl isopropanol and 50 µl MAG Suspension

It is important to mix the MAG Suspension by vigorous shaking or vortexing before use!

Tube B: 500 µl Washing Solution HS

Tube C: 800 µl Washing Solution MS

Tube D: 800 µl Washing Solution MS

Tube E: 200 µl Elution Buffer

2. After lysis transfer the lysed sample into the Tube A of the KingFisher Tube Strip, pre-filled with isopropanol and MAG Suspension
3. Place the filled KingFisher Tube Strips into the KingFisher system on the right position!
4. Place the KingFisher Tip Combs onto the magnetic track!
5. Start the program **“INNU_GDNA_KFml”!**

   Note: If you use a disc, load the program “INNU_gDNA_KFml”!

Note

1. After finishing the extraction protocol, the Tube E of the Tube Strip contains the extracted DNA. Store the DNA under adequate conditions. We recommend to store the extracted DNA at –22 °C. to -18 °C

2. If the DNA contains carryover of magnetic particles, transfer the DNA into a 1.5 ml reaction tube, centrifuge at maximum speed for 1 minute and pipette the supernatant with DNA into a new tube.

10 Protocol 2: Isolation of DNA from tissue sample (up to 30 mg or rodent tails 0.4 –1.0 cm)

A. Lysis of sample

1. Cut max. **30 mg** of **tissue sample** or **rodent tails (0.4 – 1 cm)** into small pieces and transfer the sample into a 1.5 ml or 2.0 ml reaction tube.
2. Add **400 µl Lysis Solution QPT, 20 µl Proteinase K and 3 µl RNase A** (stock solution 100 mg/ml; not included in the kit), mix vigorously by pulsed vortexing for 5 seconds.
3. Incubate at 50 °C until the sample is completely lysed (appr. 0.5 – 2 h for tissue sample and appr. 3 h for rodent tails).

   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.

Important

The lysis step should be finished if the material is completely lysed.

B. Preliminary steps of the KingFisher ml

1. During sample lysis pre-fill the tubes of the KingFisher Tube Strips with the following buffers respectively.

Tube A: 400 µl isopropanol and 50 µl MAG Suspension

It is important to mix the MAG Suspension by vigorous shaking or vortexing before use!

Tube B: 500 µl Washing Solution HS

Tube C: 800 µl Washing Solution MS

Tube D: 800 µl Washing Solution MS

Tube E: 200 µl Elution Buffer

2. After lysis centrifuge the 1.5 ml reaction tube at 10,000 x g (~12,000 rpm) for 30 seconds to spin down unlysed material.

3. Apply the sample (supernatant) to the Tube A of the KingFisher Tube Strip, pre-filled with isopropanol and MAG Suspension
4. Place the filled KingFisher Tube Strips into the KingFisher system on the right position!
5. Place the KingFisher Tip Combs onto the magnetic track!
6. Start the program **“INNU_GDNA_KFml”!**

   Note: If you use a disc, load the program “INNU_gDNA_KFml”!

Note

1. After finishing the extraction protocol, the Tube E of the Tube Strip contains the extracted DNA. Store the DNA under adequate conditions. We recommend to store the extracted DNA at -22 °C to -18 °C.
2. If the DNA contains carryover of magnetic particles, transfer the DNA into a 1.5 ml reaction tube, centrifuge at maximum speed for 1 minute and pipette the supernatant with DNA into a new tube.

11 Protocol 3: Isolation of DNA from paraffin embedded tissue samples

A. Sample Lysis

1. Place the FFPE (formalin-fixed, paraffin-embedded) material (approx. 2 x 5 µm; optional more starting material) into a 1.5 ml or 2.0 ml reaction tube.
2. Add **400 µl Lysis Solution QPT and 20 µl Proteinase K**, mix vigorously by pulsed vortexing for 5 sec. Incubate at 50 °C for 1 hour.

   Note: The FFPE material must completely covered with Lysis Solution QPT!
3. After lysis step place the sample into a thermal mixer pre-heated to 90 °C and incubate the sample for 1 h.

   Important Note: Place the sample first after achieve of the 90 °C into the thermal mixer!

B. Preliminary steps of the KingFisher ml

1. During sample lysis pre-fill the tubes of the KingFisher Tube Strips with the following buffers respectively.

Tube A: 400 µl isopropanol and 50 µl MAG Suspension

It is important to mix the MAG Suspension by vigorous shaking or vortexing before use!

Tube B: 500 µl Washing Solution HS

Tube C: 800 µl Washing Solution MS

Tube D: 800 µl Washing Solution MS

Tube E: 120 µl Elution Buffer

2. After sample lysis centrifuge the 1.5 ml reaction tube at 10.000 x g (~12.000 rpm) for 1 minute.
3. Apply the supernatant to the Tube A of the KingFisher Tube Strip, pre-filled with isopropanol and MAG Suspension

   Note: Avoid carry over of residual FFPE material!

3. Place the filled KingFisher Tube Strips into the KingFisher system on the right position!
4. Place the KingFisher Tip Combs onto the magnetic track!
5. Start the program **“INNU_GDNA_KFml”!**

   Note: If you use a disc, load the program “INNU_gDNA_KFml”!

Note

1. After finishing the extraction protocol, the Tube E of the Tube Strip contains the extracted DNA. Store the DNA under adequate conditions. We recommend storing the extracted DNA at -22 °C to -18 °C.
2. If the DNA contains carryover of magnetic particles, transfer the DNA into a 1.5 ml reaction tube, centrifuge at maximum speed for 1 minute and pipette the supernatant with DNA into a new tube.

12 Protocol 4: Isolation of DNA from buccal swab

A. Sample Lysis

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!

4. Place the **swab** into a 1.5 ml reaction tube.
5. Add **400 µl Lysis Solution QPT, 20 µl Proteinase K and optionally 3 µl RNase A** (stock solution 100 mg/ml; not included in the kit), mix vigorously by pulsed vortexing for 5 seconds.
6. Incubate at 50 °C for 10 – 15 minutes.

   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 3 – 4 times during the incubation. No shaking will reduce the lysis efficiency!
7. After lysis time remove the swab from the tube and squeeze the swab on the wall of the tube to remove all Lysis Solution QPT from the swab.

B. Preliminary steps of the KingFisher ml

1. During sample lysis pre-fill the tubes of the KingFisher Tube Strips with the following buffers respectively.

Tube A: 400 µl isopropanol and 50 µl MAG Suspension

It is important to mix the MAG Suspension by vigorous shaking or vortexing before use!

Tube B: 500 µl Washing Solution HS

Tube C: 800 µl Washing Solution MS

Tube D: 800 µl Washing Solution MS

Tube E: 150 µl Elution Buffer

2. After lysis apply the sample to the Tube A of the KingFisher Tube Strip, pre-filled with isopropanol and MAG Suspension
4. Place the filled KingFisher Tube Strips into the KingFisher system on the right position!
4. Place the KingFisher Tip Combs onto the magnetic track!
5. Start the program **“INNU_GDNA_KFml”!**

   Note: If you use a disc, load the program “INNU_gDNA_KFml”!

Note

1. After finishing the extraction protocol, the Tube E of the Tube Strip contains the extracted DNA. Store the DNA under adequate conditions. We recommend storing the extracted DNA at -22 °C to -18 °C.
2. If the DNA contains carryover of magnetic particles, transfer the DNA into a 1.5 ml reaction tube, centrifuge at maximum speed for 1 minute and pipette the supernatant with DNA into a new tube.

engl. 06/18 – Analytik Jena AG, Jena

Headquarters

Analytik Jena AG
Konrad-Zuse-Str. 1
07745 Jena · Germany

Phone +49 3641 77 70
Fax +49 3641 77 9279
info@analytik-jena.com
www.analytik-jena.com

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Subject to changes in design and scope of delivery as well as further technical development!