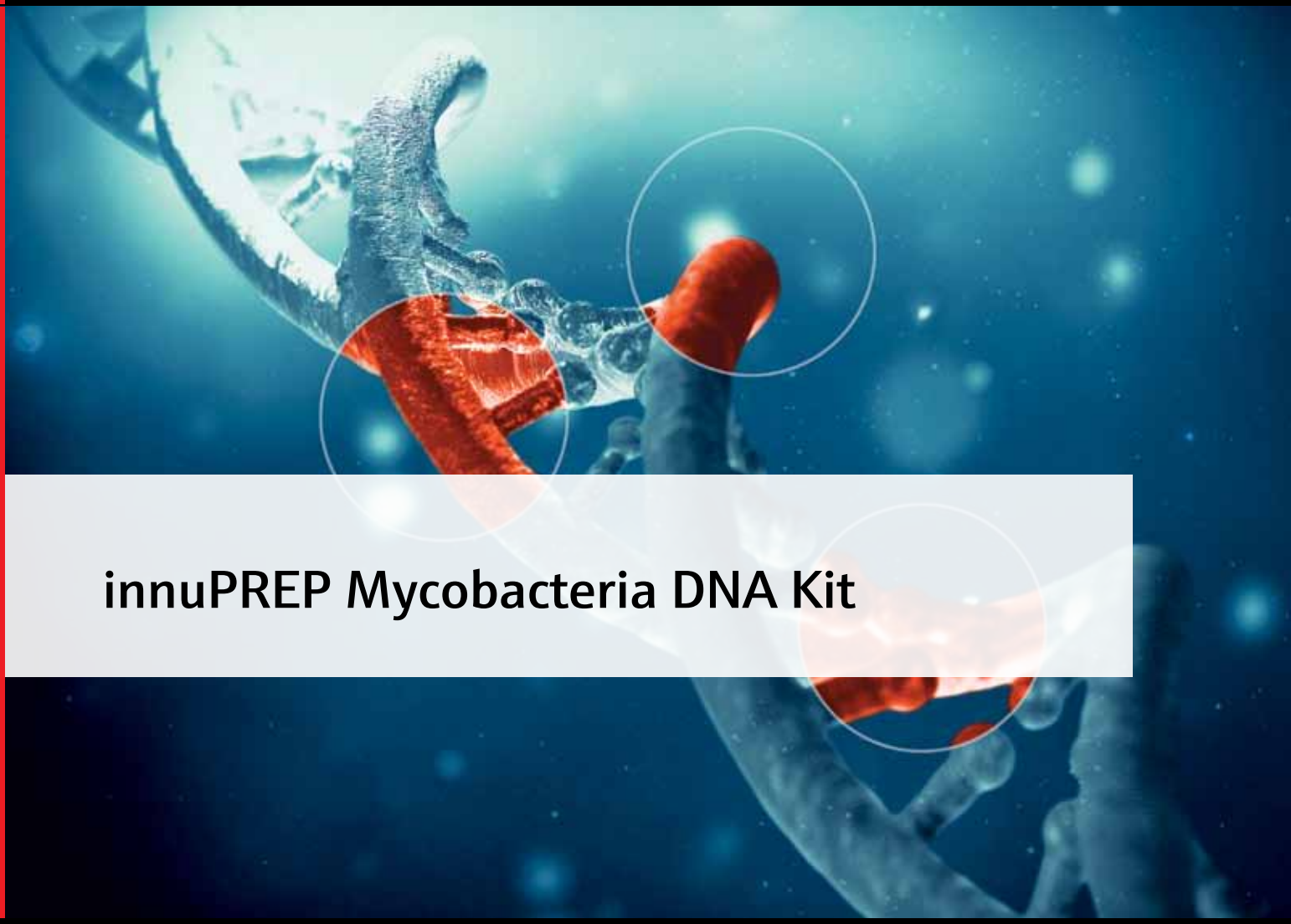
Instructions for Use
Life Science Kits & Assays



innuPREP Mycobacteria DNA Kit

analytikjena
An Endress+Hauser Company

Order No.:

845-KS-6100010	10 reactions
845-KS-6100050	50 reactions
845-KS-6100250	250 reactions

Publication No.: HB_KS-6100_e_120116

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 (0) 36 41 / 77-94 00
Fax +49 (0) 36 41 / 77-76 77 76
www.bio.analytik-jena.com
lifescience@analytik-jena.com

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 innuPREP Mycobacteria 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 innuPREP Mycobacteria DNA Kit were tested by isolation of bacterial DNA spiked in a mucous 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 innuPREP Mycobacteria 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!

	10 extractions	50 extractions	250 extractions
NAC Buffer	2 x 2 ml	15 ml	60 ml
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 ml	10 ml	30 ml
Spin Filter (blue)	10	50	5 x 50
Receiver Tubes (2.0 ml)	40	4 x 50	20 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 the Proteinase K by addition of 0.3 ml ddH_20, 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 the Proteinase K by addition of 1.5 ml ddH_20, 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 the Proteinase K by addition of 1.5 ml ddH_20, mix thoroughly and store as described below!

Important note

Dividing the dissolved Proteinase K into aliquots and storage at – 20 °C is recommended. Repeated freezing and thawing will reduce the activity dramatically.

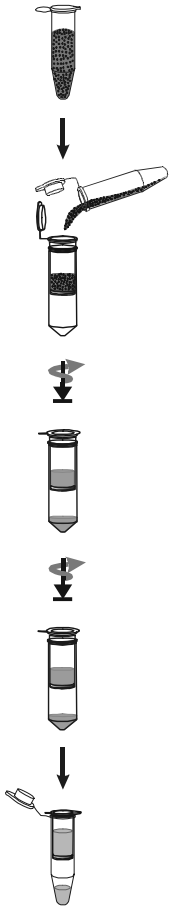
6 Recommended steps before starting

- Heat thermal mixer or water bath at 37 °C, 50 °C and 95 °C; optional 68 °C
- Ensure that the Washing Solution HS, the Washing Solution MS and the Proteinase K have been prepared according to the instruction (→ "Kit components" p. 4)
- Centrifugation steps should be carried out at room temperature

7 Components not included in the kit

- 96–99.8 % ethanol
- 1.5 ml reaction tubes
- ddH_2O
- TE Buffer
- Lysozyme (stock solution 10 mg/ml TE Buffer)

8 General procedure for DNA extraction



Lysis of the starting material

Binding of DNA onto Spin Filter (blue)

Washing of the bound DNA

Elution of DNA

9 Product specifications

1. Starting material:

- Sputum samples (0.2 – 5 ml)
- Bronchoalveolar lavage (up to 1.0 ml)
- Tissue biopsies (1.0 – max. 10 mg; e.g. lymph nodes)

2. Time for isolation:

Approximately 15 minutes after the lysis step

3. Typical yield:

Not determined. The yield depends on the type of mycobacteria and on the amount of the starting material. The typical yield is sufficient for amplification procedures.

10 Protocol 1: Isolation of mycobacteria DNA from sputum sample (200 µl sample)

Important
Heat thermal mixer to 37 °C, 95 °C and 50 °C; pre-heat the needed amount of Elution Buffer at 50 °C

Be aware of risk of infection when handling material containing MYCOBACTERIA!

1. Transfer **200 µl** of **sputum** into a 1.5 ml reaction tube and add **200 µl NAC Buffer**. Vortex shortly and incubate the sample at room temperature under continuous shaking for 20 minutes. Centrifuge the sample at 8.000 x g (~10.000 rpm) for 15 minutes. Remove the supernatant carefully, but completely.

   Don't discard the pellet!

2. Add **200 µl TE Buffer** to the bacterial pellet and re-suspend the pellet completely.
3. Add **15 µl Lysozym** (stock solution 10 mg/ml in TE buffer). Mix by pulsed vortexing for 5 s. Incubate at 37 °C for 30 minutes.

   Note: The lysis time can be increased.

   We recommend to use a shaking platform (thermal mixer, water bath or another rocking platform) for a continuous shaking of the sample. Vortex the sample optionally during incubation.
4. After lysis with Lysozyme add **200 µl Lysis Solution TLS** and incubate the tube at 95 °C for 20 minutes. After incubation at 95 °C place the tube on ice for 2 minutes.
5. Open the tube and add **25 µl Proteinase K** and incubate at 50 °C for 30 minutes.

   Note: We recommend to use a shaking platform (thermal mixer, water bath or another rocking platform) for a continuous shaking of the sample. Vortex the sample optionally during incubation. No shaking will reduce the lysis efficiency!
6. Add **400 µl Binding Solution TBS** 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 TBS are mixed vigorously to get a homogeneous solution.
7. Apply **600 µl of** the **sample** to the 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. If there is residual sample transfer it to the Spin Filter and centrifuge again.

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.

8. 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.
9. Open the Spin Filter and add **650 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.
10. Centrifuge at max. speed for 2 minutes to remove all traces of ethanol. Discard the 2.0 ml Receiver Tube.
11. Place the Spin Filter into a 1.5 ml Elution Tube. Carefully open the cap of the Spin Filter and add **50 µl pre-heated Elution Buffer**. Incubate at room temperature for 2 minutes. Centrifuge at 6.000 x g (~8.000 rpm) for 1 minute. Add **another 50 µl pre-heated Elution Buffer** to the Spin Filter. Centrifuge at 6.000 x g (8.000 rpm) for 1 minute.

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.

analytikjena

innuPREP Mycobacteria DNA Kit

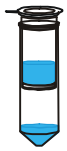
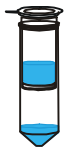
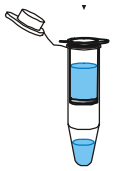
Protocol 1: Isolation of mycobacteria DNA from sputum sample

Recommended steps before starting

- Heat thermal mixer or water bath (37 °C, 95 °C and 50 °C)
- Pre-heat the needed amount of Elution Buffer (50 °C)
- Prepare Washing Solution HS, Washing Solution MS and Proteinase K according to the instruction

1.	Starting material	▪ Sputum sample		▪ 200 µl
2.	Pellet cells			▪ Add 200 µl NAC Buffert, vortex ▪ Incubation: 20 min @ RT ▪ 8.000 x g (~10.000 rpm): 15 min ▪ Remove supernatant
3.	Re-suspend			▪ Add 200 µl TE buffer ▪ Re-suspend cell pellet completely
4.	Lysis			▪ Add 15 µl Lysozym ▪ Vortex: 5 sec; pulsed ▪ Incubation: 37 °C, 30 min ▪ Add 200 µl TLS ▪ Incubation: 95 °C, 20 min ▪ Place tube on ice: 2 min ▪ Add 25 µl PK ▪ Incubation: 50 °C, 30 min
5.	Binding of DNA		 	▪ Add 400 µl TBS, vortex ▪ Add Spin Filter to Receiver Tube ▪ Add 600 µl sample to Spin Filter ▪ 10.000 x g (~12.000 rpm): 2 min ▪ Load residual sample ▪ 10.000 x g (~12.000 rpm): 2 min

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

6.	Washing New Receiver Tube		▪ Add 500 µl HS ▪ 10.000 x g (~12.000 rpm): 1 min ▪ Add 650 µl MS ▪ 10.000 x g (~12.000 rpm): 1 min
7.	Remove Ethanol New Receiver Tube		▪ Discard filtrate ▪ Add Spin Filter to Receiver Tube ▪ Centrifuge: max speed, 2 min ▪ Add Spin Filter to an Elution Tube
8.	Elution (Repeat 2x)		▪ Add 50 µl pre-heat Elution Buffer ▪ Incubation: 2 min @ RT ▪ 6.000 x g (~8.000 rpm): 1 min

Order No.:

845-KS-6100010	10 reactions
845-KS-6100050	50 reactions
845-KS-6100250	250 reactions

This documentation describes the state at the time of publishing. It needs not necessarily agree with future versions. Subject to change!

Manufacturer:
AJ Innuscreen GmbH
Robert-Rössle-Straße 10
13125 Berlin

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

Phone +49 (0) 36 41 / 77-94 00
Fax +49 (0) 36 41 / 77-76 77 76
www.bio.analytik-jena.com
lifescience@analytik-jena.com

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

analytikjena

innuPREP Mycobacteria DNA Kit

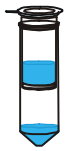
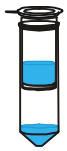
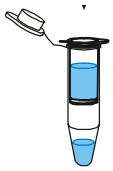
Protocol 2: Isolation of mycobacteria DNA from bronchoalveolar lavage sample

Recommended steps before starting

- Heat thermal mixer or water bath (37 °C, 95 °C and 50 °C)
- Pre-heat the needed amount of Elution Buffer (50 °C)
- Prepare Washing Solution HS, Washing Solution MS and Proteinase K according to the instruction

1.	Starting material	▪ Bronchoalveolar lavage		▪ Up to 1.0 ml
2.	Pellet cells			▪ 8.000 x g (~10.000 rpm): 15 min ▪ Remove supernatant
3.	Re-suspend			▪ Add 200 µl TE buffer ▪ Re-suspend cell pellet completely
4.	Lysis			▪ Add 15 µl Lysozym ▪ Vortex: 5 sec; pulsed ▪ Incubation: 37 °C, 30 min ▪ Add 200 µl TLS ▪ Incubation: 95 °C, 20 min ▪ Place tube on ice: 2 min ▪ Add 25 µl PK ▪ Incubation: 50 °C, 30 min
5.	Binding of DNA		 	▪ Add 400 µl TBS, vortex ▪ Add Spin Filter to Receiver Tube ▪ Add 600 µl sample to Spin Filter ▪ 10.000 x g (~12.000 rpm): 2 min ▪ Load residual sample ▪ 10.000 x g (~12.000 rpm): 2 min

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

6.	Washing New Receiver Tube		▪ Add 500 µl HS ▪ 10.000 x g (~12.000 rpm): 1 min ▪ Add 650 µl MS ▪ 10.000 x g (~12.000 rpm): 1 min
7.	Remove Ethanol New Receiver Tube		▪ Discard filtrate ▪ Add Spin Filter to Receiver Tube ▪ Centrifuge: max speed, 2 min ▪ Add Spin Filter to an Elution Tube
8.	Elution (Repeat 2x)		▪ Add 50 µl pre-heat Elution Buffer ▪ Incubation: 2 min @ RT ▪ 6.000 x g (~8.000 rpm): 1 min

Order No.:

845-KS-6100010	10 reactions
845-KS-6100050	50 reactions
845-KS-6100250	250 reactions

This documentation describes the state at the time of publishing. It needs not necessarily agree with future versions. Subject to change!

Manufacturer:
AJ Innuscreen GmbH
Robert-Rössle-Straße 10
13125 Berlin

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

Phone +49 (0) 36 41 / 77-94 00
Fax +49 (0) 36 41 / 77-76 77 76
www.bio.analytik-jena.com
lifescience@analytik-jena.com

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

analytikjena

innuPREP Mycobacteria DNA Kit

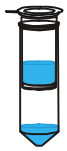
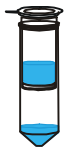
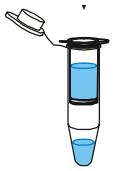
Protocol 3: Isolation of mycobacteria DNA from sputum sample

Recommended steps before starting

- Heat thermal mixer or water bath (68 °C, 37 °C, 95 °C, 50 °C)
- Pre-heat the needed amount of Elution Buffer (50 °C)
- Prepare Washing Solution HS, Washing Solution MS and Proteinase K according to the instruction
- Order additional NAC Buffer

1.	Starting material	▪ Sputum sample	▪ Up to 5.0 ml
2.	Inactivation	▪ 15 ml reaction tube	▪ Incubation: 68 °C, 30 min
3.	Pellet cells	▪ 15 ml reaction tube	▪ Add equal volume NAC Buffer ▪ Vortex ▪ Incubation: 20 min @ RT ▪ 2.500 x g (~3.000 rpm): 15 min ▪ Remove supernatant
4.	Re-suspend	▪ 15 ml reaction tube	▪ Add 200 µl TE buffer ▪ Re-suspend cell pellet completely
5.	Lysis	▪ 15 ml reaction tube	▪ Add 15 µl Lysozym ▪ Vortex: 5 sec; pulsed ▪ Incubation: 37 °C, 30 min ▪ Add 200 µl TLS ▪ Incubation: 95 °C, 20 min ▪ Place tube on ice: 2 min ▪ Add 25 µl PK ▪ Incubation: 50 °C, 30 min
6.	Binding of DNA		▪ Add 400 µl TBS, vortex ▪ Add Spin Filter to Receiver Tube ▪ Add 600 µl sample to Spin Filter ▪ 10.000 x g (~12.000 rpm): 2 min ▪ Load residual sample ▪ 10.000 x g (~12.000 rpm): 2 min

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

7.	Washing New Receiver Tube		▪ Add 500 µl HS ▪ 10.000 x g (~12.000 rpm): 1 min ▪ Add 650 µl MS ▪ 10.000 x g (~12.000 rpm): 1 min
8.	Remove Ethanol New Receiver Tube		▪ Discard filtrate ▪ Add Spin Filter to Receiver Tube ▪ Centrifuge: max speed, 2 min ▪ Add Spin Filter to an Elution Tube
9.	Elution (Repeat 2x)		▪ Add 50 µl pre-heat Elution Buffer ▪ Incubation: 2 min @ RT ▪ 6.000 x g (~8.000 rpm): 1 min

Order No.:	845-KS-6100010	10 reactions
	845-KS-6100050	50 reactions
	845-KS-6100250	250 reactions

This documentation describes the state at the time of publishing. It needs not necessarily agree with future versions. Subject to change!

Manufacturer:
AJ Innuscreen GmbH
Robert-Rössle-Straße 10
13125 Berlin

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

Phone +49 (0) 36 41 / 77-94 00
Fax +49 (0) 36 41 / 77-76 77 76
www.bio.analytik-jena.com
lifescience@analytik-jena.com

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

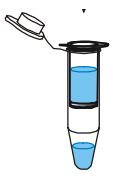
analytikjena

innuPREP Mycobacteria DNA Kit

Protocol 4: Isolation of mycobacteria DNA from tissue biopsies

Recommended steps before starting

- Heat thermal mixer or water bath (95 °C and 50 °C)
- Pre-heat the needed amount of Elution Buffer (50 °C)
- Prepare Washing Solution HS, Washing Solution MS and Proteinase K according to the instruction

1.	Starting material	- E.g. lymph nodes		- 1.0 – 10 mg
2.	Lysis			- Add 400 µl TLS and 25 µl PK - 20 µl Lysozym - Vortex: shortly - Incubation 1: 50 °C, 30 – 60 min - Incubation 2: 95 °C, 30 min
3.	Binding of DNA		 	- Add 400 µl TBS, vortex - Add Spin Filter to Receiver Tube - Add 600 µl sample to Spin Filter - 10.000 x g (~12.000 rpm): 2 min - Load residual sample - 10.000 x g (~12.000 rpm): 2 min
4.	Washing New Receiver Tube			- Add 500 µl HS - 10.000 x g (~12.000 rpm): 1 min - Add 650 µl MS - 10.000 x g (~12.000 rpm): 1 min
5.	Remove Ethanol New Receiver Tube			- Discard filtrate - Add Spin Filter to Receiver Tube - Centrifuge: max speed, 2 min - Add Spin Filter to an Elution Tube
6.	Elution (Repeat 2x)			- Add 50 µl pre-heat Elution Buffer - Incubation: 2 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 ✂

analytikjena

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

Order No.:	845-KS-6100010	10 reactions
	845-KS-6100050	50 reactions
	845-KS-6100250	250 reactions

This documentation describes the state at the time of publishing. It needs not necessarily agree with future versions. Subject to change!

Manufacturer:
AJ Innuscreen GmbH
Robert-Rössle-Straße 10
13125 Berlin

Distribution/Publisher:
Analytik Jena AG
Konrad-Zuse-Straße 1
07745 Jena/ Germany
Phone +49 (0) 36 41 / 77-94 00
Fax +49 (0) 36 41 / 77-76 77 76
www.bio.analytik-jena.com
lifescience@analytik-jena.com

11 Protocol 2: Isolation of mycobacteria DNA from bronchoalveolar lavage sample

Important
Heat thermal mixer to 37 °C, 95 °C and 50 °C; pre-heat the needed amount of Elution Buffer at 50 °C

Be aware of risk of infection when handling material containing MYCOBACTERIA!

1. Transfer up to **1 ml** of **bronchalveolar lavage** sample into a reaction tube and centrifuge the sample at 8.000 x g (~10.000 rpm) for 15 minutes. Remove the supernatant carefully but completely.

   Don't discard the pellet!

2. Add **200 µl TE buffer** to the bacterial pellet and re-suspend the pellet completely.
3. Add **15 µl Lysozym** (stock solution 10 mg/ml in TE buffer). Mix by pulsed vortexing for 5 sec. Incubate at 37 °C for 30 minutes.

   Note: The lysis could require more time.

   We recommend to use a shaking platform (thermal mixer, water bath or another rocking platform) for a continuous shaking of the sample. Vortex the sample optionally during incubation.
4. After lysis with Lysozyme add **200 µl Lysis Solution TLS** and incubate the tube at 95 °C for 20 minutes. After incubation at 95 °C place the tube on ice for 2 minutes.
5. Open the tube and add **25 µl Proteinase K** and incubate at 50 °C for 30 minutes.

   Note: We recommend to use a shaking platform (thermal mixer, water bath or another rocking platform) for a continuous shaking of the sample. Vortex the sample optionally during incubation. No shaking will reduce the lysis efficiency!
6. Add **400 µl Binding Solution TBS** 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 TBS are mixed vigorously to get a homogeneous solution.
7. Apply **600 µl of** the **sample** to the 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. If there is residual sample transfer it to the Spin Filter and centrifuge again.

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.

8. 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.
9. Open the Spin Filter and add **650 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.
10. Centrifuge at max. speed for 2 minutes to remove all traces of ethanol. Discard the 2.0 ml Receiver Tube.
11. Place the Spin Filter into a 1.5 ml Elution Tube. Carefully open the cap of the Spin Filter and add **50 µl pre-heated Elution Buffer**. Incubate at room temperature for 2 minutes. Centrifuge at 6.000 x g (~8.000 rpm) for 1 minute. Add **another 50 µl pre-heated Elution Buffer** to the Spin Filter. Centrifuge at 6.000 x g (~8.000 rpm) for 1 minute.

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 Protocol 3: Isolation of mycobacteria DNA from sputum sample of larger volumes (up to 5 ml)

Important
Heat thermal mixer to 37 °C, 95 °C and 50 °C; pre-heat the needed amount of Elution Buffer at 50 °C
Order additional NAC Buffer

Be aware of risk of infection when handling material containing MYCOBACTERIA!

1. Transfer **up to 5 ml** of **sputum** sample into a 15 ml reaction tube. Inactivate the sample by incubation at 68 °C for 30 minutes. Add an **equal volume of NAC Buffer**, vortex shortly and incubate the sample at room temperature for 20 minutes. Centrifuge at 2.500 x g (3.000 rpm) for 15 minutes. Remove the supernatant carefully but completely.

   Don't discard the pellet!

2. Add **200 µl TE buffer** to the bacterial pellet and re-suspend the pellet completely.
3. Add **15 µl Lysozym** (stock solution 10 mg/ml in TE buffer). Mix by pulsed vortexing for 5 sec. Incubate at 37 °C for 30 minutes.

   Note: The lysis could require more time.

   We recommend to use a shaking platform (thermal mixer, water bath or another rocking platform) for a continuous shaking of the sample. Vortex the sample optionally during incubation.
4. After lysis with Lysozyme add **200 µl Lysis Solution TLS** and incubate the tube at 95 °C for 20 minutes. After incubation at 95 °C place the tube on ice for 2 minutes.
5. Open the Tube and add **25 µl Proteinase K** and incubate at 50 °C for 30 minutes.

   Note: We recommend to use a shaking platform (thermal mixer, water bath or another rocking platform) for a continuous shaking of the sample. Vortex the sample optionally during incubation. No shaking will reduce the lysis efficiency!
6. Add **400 µl Binding Solution TBS** 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 TBS are mixed vigorously to get a homogeneous solution.

7. Apply **600 µl of** the **sample** to the 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. If there is residual sample transfer it to the Spin Filter and centrifuge again.

   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.

8. 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.

9. Open the Spin Filter and add **650 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.

10. Centrifuge at max. speed for 2 minutes to remove all traces of ethanol. Discard the 2.0 ml Receiver Tube.

11. Place the Spin Filter into a 1.5 ml Elution Tube. Carefully open the cap of the Spin Filter and add **50 µl pre-heated Elution Buffer**. Incubate at room temperature for 2 minutes. Centrifuge at 6.000 x g (~8.000 rpm) for 1 minute. Add **another 50 µl pre-heated Elution Buffer** to the Spin Filter. Centrifuge at 6.000 x g (~8.000 rpm) for 1 minute.

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.

13 Protocol 4: Isolation of mycobacteria DNA from tissue biopsies (e.g. lymph nodes)

Important
Heat heating blocks (e.g. thermal mixer) to 50 °C and 95 °C; pre-heat the needed amount of Elution Buffer at 50 °C

Be aware of risk of infection when handling material containing MYCOBACTERIA!

1. Transfer **1 mg up to – max. 10 mg** of the **biopsy** into a 1.5 ml tube. Add **400 µl of Lysis Solution TLS, 25 µl Proteinase K and 20 µl Lysozyme** (stock solution 10 mg/ml). Close the cap and vortex shortly. Place the tube into a thermal mixer and incubate under continuously shaking for 30 – 60 minutes at 50 °C.

   Note: Lysis time can be increased if the lysis is not completed.

2. Place the tube into a thermal mixer and incubate at 95 °C for 30 minutes under continuous shaking (continuous shaking increases the lysis efficiency).

3. Add **400 µl Binding Solution TBS** 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 TBS are mixed vigorously to get a homogeneous solution.

4. Apply **600 µl of** the **sample** to the 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. If there is residual sample transfer it to the Spin Filter and centrifuge again.

   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.

5. 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.

6. Open the Spin Filter and add **650 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.

7. Centrifuge at max. speed for 2 minutes to remove all traces of ethanol. Discard the 2.0 ml Receiver Tube.

8. Place the Spin Filter into a 1.5 ml Elution Tube. Carefully open the cap of the Spin Filter and add **50 µl pre-heated Elution Buffer**. Incubate at room temperature for 2 minutes. Centrifuge at 6.000 x g (~8.000 rpm) for 1 minute. Add another **50 µl pre-heated Elution Buffer** to the Spin Filter. Centrifuge at 6.000 x g (~8.000 rpm) for 1 minute.

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.

14 Troubleshooting

Problem / probable cause	Comments and suggestions
Clogged Spin-Filter	Increase lysis time. Increase centrifugation speed or time. Reduce amount of starting material.
Low amount of extracted DNA	
• Insufficient lysis	Increase lysis time at 95 °C. Don't forget the addition of Proteinase K!
• Incomplete elution	Prolong the incubation time with Elution Buffer to 5 minutes or repeat elution step once again. Take higher volume of Elution Buffer.
• Insufficient mixing with Binding Solution TBS	Mix sample with Binding Solution TBS completely by pipetting or by vortexing prior 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.
No amplification results	
• Incorrect storage of starting material	Ensure that the starting material is fresh or has been stored under appropriate conditions (for long-term storage at –20 °C)! Avoid repeated thawing and freezing of the material.
• Old material	Old material often contains degraded DNA.
• Ethanol carryover during elution	Increase time for removing of ethanol.
• Salt carryover during elution	Ensure that Washing Solution is at room temperature. Check up Washing Solution for salt precipitates. If there are any precipitates, solve these precipitates by careful warming.

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

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