EXTRACTME®
DNA BACTERIA KIT

Cat. No. EM02
Version: 1.2018

Kit for genomic DNA isolation from bacteria

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I. INTENDED USE

The ***EXTRACTME*** **DNA BACTERIA KIT** is designed for a rapid and efficient purification of high quality bacterial gDNA from broth and plate cultures as well as frozen cells. The isolation protocol and buffer formulations were optimized for high isolation efficiency and DNA purity. The product is intended for research use only.

II. COMPONENTS OF THE KIT AND STORAGE CONDITIONS

NUMBER OF ISOLATIONS	10 ISOLATIONS	50 ISOLATIONS	250 ISOLATIONS	STORAGE CONDITIONS[1]
Catalogue number	EM02-010	EM02-050	EM02-250	
BacL Buffer (Lysis Buffer)	3 ml	15 ml	75 ml	RT
⚠ **RNase A**[*] (lyophilized)	1 tube	1 tube	5 tubes	-20°C[2]
RNase Buffer	100 µl	220 µl	1.1 ml	RT
⚠ **Proteinase K**[**] (lyophilized)	1 tube	1 tube	5 tubes	-20°C[3]
Proteinase Buffer	200 µl	940 µl	4.7 ml	RT
BacB Buffer (Binding Buffer)	3.5 ml	18 ml	88 ml	RT
BacW Buffer (Wash Buffer)	10 ml	50 ml	250 ml	RT
Elution Buffer	2 ml	10 ml	5 x 10 ml	RT
DNA Purification Columns	10 pcs	50 pcs	5 x 50 pcs	RT
Collection Tubes (2 ml)	10 pcs	50 pcs	5 x 50 pcs	RT

[*] Prior to the first use, add 220 µl RNase Buffer to a tube containing RNase A lyophilizate (in the kit for 10 isolations 100 µl of a buffer should be added).

[**] Prior to the first use, add 940 µl Proteinase Buffer to a tube containing Proteinase K lyophilizate (in the kit for 10 isolations 200 µl of a buffer should be added). In order to avoid evaporation, ensure that the buffer bottles are tightly closed before storing.

[1] RT – room temperature (+15°C to +25°C)

[2] After reconstitution, **RNase A** should be kept at **+4°C for short term storage (several days) or in aliquots at -20°C**.

[3] After reconstitution, **Proteinase K** should be kept at **-20°C**.

Expiry date

Under proper storage conditions the kit will remain stable for at least 12 months from opening or until the expiry date.

III. ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

- sterile microcentrifuge tubes (1.5–2 ml)
- automatic pipettes and sterile DNase-free tips
- microcentrifuge (≥ 11 000 x g)
- thermal heating block or water bath (up to 55°C)
- vortex mixer
- disposable gloves

Might be necessary:

- TE buffer
- lysostaphin
- lysozyme

IV. PRINCIPLE

DNA purification procedure consists of five steps and utilizes spin minicolumns with membranes which efficiently and selectively bind nucleic acids. During the first step, cell walls, membranes and proteins are degraded by lysis buffer and Proteinase K. To obtain an RNA-free DNA sample, RNA is removed by RNase A. After addition of chaotropic salts, lysate is applied to purification column membrane and DNA is bound. A two-step washing stage effectively removes impurities and enzyme inhibitors. Purified DNA is eluted with the use of a low ionic strength buffer (Elution Buffer) or water (pH 7.0–9.0) and may be used directly in all downstream applications such as PCR, qPCR, Southern blotting, DNA sequencing, enzymatic restriction, DNA ligation and so forth, or stored until ready to use.

V. QUALITY CONTROL

The quality of each production batch (LOT) of ***EXTRACTME*** **DNA BACTERIA KIT** is tested using standard QC procedures. Purified DNA concentration and quality are evaluated by gel electrophoresis and spectrophotometer.

VI. PRODUCT SPECIFICATIONS

SAMPLE MATERIAL
Broth or plate bacterial culture, frozen cell pellet.
EFFICIENCY
up to 5 x 10^8 cells → 100% 10^9 ÷ 3 x 10^9 → 75-90% (when the modified protocol for isolation from a great number of cells is followed) 10^9 ÷ 3x 10^9 → ≤ 60% (when the standard isolation protocol is followed)
BINDING CAPACITY
Approx. 40 µg DNA
TIME REQUIRED
Approx. 40–60 minutes (including incubation time)
DNA PURITY
A_{260}/A_{280} ratio = 1.7–1.9

VII. SAFETY PRECAUTIONS

- Bacterial culture is treated as a biohazardous material for its potential pathogen content or health and life-threatening substances. While working with bacterial cultures, it is essential to comply with all safety requirements for working with biohazardous material.
- It is advisable to conduct the entire isolation procedure in a Class II Biological Safety Cabinet or at laboratory burner as well as wearing disposable gloves and suitable lab coat at all times.
- The use of sterile filter tips is recommended.
- Avoid cross-contamination between sample preparations.

- → Guanidine salts' can form highly reactive compounds when combined with bleach or other oxidation components. In case of spillage, clean the surface with suitable laboratory detergent and water.
- → In case of spillage of liquid containing microorganisms, clean the contaminated surface with detergent-water solution.

VIII. RECOMMENDATIONS AND IMPORTANT NOTES

Sample material

DNA isolation efficiency and purity can depend not only on number of bacterial cells, cell type (morphology) or antibiotics in grow medium, but also on age and condition of the cells.

The kit is designed to isolate DNA from max. 3×10^9 bacterial cells. Using a greater number of cells may significantly reduce isolation efficiency and purity of DNA.

Extracting DNA from fresh starting material or frozen cell pellets is recommended. To minimize DNA degradation, avoid repeated freeze/thaw cycles of samples. Using old or repeatedly frozen/thawed material may result in low efficiency isolation of high molecular DNA.

Interactions with the BacB Buffer

A few bacterial species may form a thick coat of slime after binding buffer has been added to lysate. Should this happen, incubate at 55°C and break the coat by vigorous vortexing or pipetting. However, extending the incubation time up to additonal ten minutes, at 55°C with the subsequent vortexing may be also plausible. If the coat is still noticeable centrifuge the lysate according to the protocol and (avoiding the coat) transfer a supernatant onto a spin minicolumn membrane and continue the isolation step 11 of the isolation protocol (section XI). Procedure should not reduce either isolation efficiency or the purity of DNA.

DNA elution

Optimal volume of elution buffer used should be chosen in line with quantity of sample material and final DNA concentration expected. Use of 50-100 µl Elution Buffer is recommended when extracting from no more than 10^9 cells. However, while extracting from a greater number of cells, the volume of elution buffer should be increased to 200 µl.

If a high DNA concentration is desired, elution volume may be reduced to 20 µl. It should be noted that this may reduce efficiency. It is essential to apply elution buffer precisely to the centre of membrane.

In order to maximize DNA retrieval, heat Elution Buffer to 80°C and incubate it on membrane for 10 minutes.
If full DNA retrieval is required, a second elution should be performed. For the second elution, repeat steps 19–22 of the Isolation Protocol (section XI), placing purification column in a new, sterile 1.5 ml Eppendorf tube.

Elution Buffer

DNA can alternatively be eluted from membrane with nuclease-free water, pH 7.0-9.0, or 5-10 mM of Tris Buffer, pH 8.0-9.0.

IX. SAMPLE PREPARATION

Isolation from broth culture (0.2-3 ml)

Before starting, always mix broth culture thoroughly. Transfer a desired quantity of bacterial culture (no more than 1.5 ml) to a sterile 1.5–2 ml Eppendorf tube and spin down the cells at 3000–4000 x g. Remove supernatant. While isolating from a greater then 1.5 ml number of cultures, add an extra 1.5 ml of the culture to the top of the cell pellet obtained this way and spin it again. Continue isolation following the Isolation Protocol from step 2 (section XI).

Isolation from more cells than recommended

When extracting DNA from a large number of cells (≥10^9), after spinning the culture, resuspend the cell pellet obtained this way in **450 µl of BacL Buffer** and **6 µl of RNaseA** (step 3), and subsequently **15 µl of Proteinase K** (step 5). Continue isolation following the Isolation Protocol from step 6 (section XI).

Isolation from frozen bacterial cells

Immediately after retrieving frozen cell pellet from freezer, resuspend it in **300 µl of BacL Buffer**. Do not allow cell pellet to thaw. Continue isolation following the Isolation Protocol from step 3 (section XI).

Isolation from plate culture

Transfer **300 µl of BacL Buffer** to a sterile 1.5 ml Eppendorf tube. Using a loop, take a sufficient amount of culture from plate and suspend it in BacL Buffer. Continue isolation following the Isolation Protocol from step 3 (section XI).

Isolation from Gram-positive bacteria

Gram-positive bacteria must be treated with an appropriate enzyme before starting the isolation. For DNA isolation from *Staphylococcus* use lysostaphin and from *Enterococcus* use lysozyme.

Staphylococcus:

1. Pellet cells by centrifugation of 1.5 ml of broth culture*.
2. Discard the supernatant and suspend the cell pellet in **200 µl TE****. Mix thoroughly.
3. Add **30 µl lysostaphin 400 U/ml** solution and **4 µl RNase A**. Mix well by pipetting or vortexing.
4. Incubate at **37°C** for 20–60 min*** (until sample is lysed).
5. Add **300 µl BacL Buffer** and **17 µl Proteinase K**. Mix thoroughly.
6. Continue the isolation following the Isolation Protocol from step 6 (section XI).

* For thick cultures, use less broth cell culture.
** TE Buffer: 10 mM Tris-HCl, 1 mM EDTA, pH 8.0.
*** When isolating from coagulase-negative strains, use 50 µl of lysostaphin and incubate 1h at 37°C.

Enterococcus:

1. Pellet cells by centrifugation of 1,5 ml of broth culture*.
2. Discard the supernatant and suspend the cell pellet in **200 µl TE****, mix thoroughly.
3. Add **40 µl lysozyme 100 mg/ml** solution and **4 µl RNase A**, mix well by pipetting or vortexing.
4. Incubate at **37°C** for 40–60 minutes (until sample is lysed).
5. Add **300 µl BacL Buffer** and **17 µl Proteinase K**, mix thoroughly.
6. Continue the isolation following the Isolation Protocol from step 6 (section XI).

* In case of thick cultures, use less broth cell culture.
** TE Buffer: 10 mM Tris-HCl, 1 mM EDTA, pH 8.0.

X. PRIOR TO ISOLATION

1. Mix well each buffer supplied with the kit.
2. Prepare **Proteinase K** solution by reconstituting lyophilizate in an appropriate quantity of Proteinase Buffer. Reconstitute **RNase A** lyophilizate in an appropriate amount of RNase Buffer.
3. Examine buffers. If a sediment has occurred in any of them, incubate it at 50–60°C (other buffers), mixing occasionally until the sediment has dissolved. Cool to room temperature.
4. Set a dry block heater or water bath to 37°C and 55°C.
5. Unless otherwise stated, conduct all the isolation steps at room temperature.

XI. ISOLATION PROTOCOL

1. Pellet cells from **0.2–1.5 ml** bacterial broth culture by centrifugation for 5 min at 3000–4000 x g.
   ⚠ If more bacterial culture is to taken for DNA isolation, follow the instructions in section IX. Sample preparation.
2. Discard supernatant and resuspend cell pellet thoroughly in **300 µl BacL Buffer**.
3. Add **4 µl RNase A** and mix well by vortexing.
4. Incubate at **37°C** for 10 min.
5. Add **10 µl Proteinase K** and mix by vortexing.
6. Incubate at **55°C** for 10 min.
7. Add **350 µl BacB Buffer** and mix thoroughly.
8. Incubate for an additional 5 min at **55°C**.
9. After incubation vortex vigorously for 15 s.
10. Centrifuge for 120 s at 11 000–15 000 x g.
11. Carefully transfer **supernatant** containing DNA into a purification minicolumn placed in a collection tube. Centrifuge for 60 s at 11 000–15 000 x g.
    ⚠ Keep pipette tip away from pellet.
12. Transfer purification minicolumn to a new collection tube (2 ml).
13. Add **600 µl BacW Buffer** and centrifuge for 30 s at 11 000–15 000 x g.

14. Discard filtrate and reuse collection tube.

15. Add **400 µl BacW Buffer** and centrifuge for 30 s at 11 000–15 000 x g.

16. Discard filtrate and reuse collection tube.

17. Centrifuge for 60–120 s at 15 000–21 000 x g.

    ⚠ BacW Buffer contains alcohol, which may interfere with some enzymatic reactions and also decrease elution efficiency. It is therefore vital to remove alcohol completely from column prior to the elution.

18. Discard collection tube and filtrate and carefully transfer purification column to a sterile 1.5 ml Eppendorf microcentrifuge tube.

19. Add **50-100 µl Elution Buffer** directly onto purification minicolumn membrane.

    ⚠ Other buffer volumes between 20–200 µl range may be used. For instructions, see section VIII. Recommendations and important notes.

20. Incubate the minicolumn at room temperature for 120 s.

21. Centrifuge at 11 000–15 000 x g for 60 s.

22. Remove minicolumn. Isolated DNA is ready for use in downstream applications or for either short-term storage at **+4°C** or long-term storage at **-20°C**.

XII. TROUBLESHOOTING

Problem	Possible cause	Solution
Incomplete cell lysis.	Too many cells were taken for DNA purification.	Reduce amount of starting material or follow instructions for isolation from more than recommended cells (section IX. Sample preparation).
	Gram-positive strain was taken for DNA isolation.	Follow instructions regarding DNA isolation from Gram-positive bacteria (section IX. Sample preparation).
	Other than bacterial strain was taken for DNA isolation.	Confirm genus identification of strain and use an appropriate DNA isolation kit.
	Insufficient lysis time for amount of material taken for isolation.	Extend incubation time at 55°C to 20 min or until cells are completely lysed.
After incubation with BacB Buffer, a thick coat of slime occurs near surface of the lysate.	Characteristic feature of bacterial strain used for isolation.	See „Interactions with BacB Buffer" in section VIII. Recommendation and important notes.
Column becomes clogged during purification.	Incomplete cell lysis.	See „Incomplete cell lysis".
	A thick slime of DNA was formed.	Repeat centrifugation for 1 min at 15k x g or at maximum speed.
Low yield of purified DNA.	Starting material contained few bacterial cells.	Increase amount of starting material or decrease volume of Elution Buffer to 20 µl.
	Incomplete cell lysis.	See the Problem „Incomplete cell lysis".
	Reduced Proteinase K activity.	Prepare a fresh Proteinase K solution. Ensure that Proteinase K solution is stored at -20°C.
	Lysate was not mixed sufficiently with binding buffer BacB.	Repeat isolation, paying a particular attention to whether the lysate is thoroughly mixed with BacB Buffer before incubating at 55°C (step 8).
	Purified DNA contains residual alcohol.	Repeat isolation, paying a particular attention to whether any residual BacW Buffer is left in the purification minicolumn after final centrifugation step.
	Incomplete DNA elution from membrane.	Before applying Elution Buffer to membrane, heat it to 80°C. Apply Elution Buffer directly to centre of membrane. Extend incubation time with Elution Buffer to 10 min. Perform second elution. Increase volume of Elution Buffer to 200 µl.
	pH of the water used for elution is lower than 7.0.	Use Elution Buffer for DNA elution.

Problem	Possible cause	Solution
Low concentration of purified DNA.	Too much Elution Buffer was used.	Decrease volume of Elution Buffer. For details, see section VIII. Recommendations and important notes.
Isolated DNA is of poor purity.	Incomplete protein degradation.	Prepare a fresh Proteinase K solution. Ensure that Proteinase K solution is stored at -20°C.
	One of washing steps was omitted.	Repeat isolation, performing both washing steps.
	Purified DNA contains residual alcohol.	Repeat isolation, paying a particular attention to ensuring that no residual BacW Buffer is left in purification minicolumn after final centrifugation step.
DNA flows out of lanes in agarose gel.	Purified DNA contains residual alcohol.	Repeat isolation, paying a particular attention to whether any residual BacW Buffer is left in the purification minicolumn after final centrifugation step.
Purified DNA is degraded.	Old or damaged material was used.	It is recommended to perform an isolation from fresh overnight bacterial broth culture. Older bacterial cultures may contain degraded DNA.
	Inappropriate sample storage conditions.	Always perform an isolation from fresh or frozen material. Avoid subjecting the sample material to repeated freeze/thaw cycles.
RNA contamination present.	Reduced RNase A activity.	Prepare a fresh RNase A solution and repeat isolation. Ensure proper storage conditions.
	Insufficient incubation time with RNase A.	Extend incubation time with RNase A (step 4) to 30 min.
Inhibition of downstream enzymatic reactions.	Purified DNA contains residual alcohol.	After each washing step, ensure that filtrate is removed from collection tube. After second washing step, empty collection tube and re-spin it for 60 seconds at the maximum speed.
	Insufficient DNA in sample material.	See „Incomplete cell lysis" and „Low concentration of purified DNA".
	High RNA contamination.	See „RNA contamination present".

XIII. SAFETY INFORMATION

Proteinase (lyophilized)

Danger
H334, H315, H319, H335
P261, P271, P342+P311, P304+P340, EUH208

BacB Buffer

Danger
H318, H315,H412
P264, P280, P305+P351+P338 P310

BacW Buffer

Danger
H225, H319, H336
P210, P264, P370+P378, P304+P340

EUH208 Contains Proteinase. May produce an allergic reaction. **H225** Highly flammable liquid and vapour. **H315** Causes skin irritation. **H318** Causes serious eye damage. **H319** Causes serious eye irritation. **H334** May cause allergy or asthma symptoms or breathing difficulties if inhaled. **H335** May cause respiratory irritation. **H336** May cause drowsiness or dizziness. **H412** Harmful to aquatic life with long-lasting effects. **P210** Keep away from heat/sparks/open flames/hot surfaces. No smoking. **P261** Avoid breathing dust/fumes/gas/mist/vapours/spray. **P264** Wash hands thoroughly after handling. **P271** Use only outdoors or in a well-ventilated area. **P280** Wear protective gloves/ protective clothing/eye protection/face protection. **P342+P311** If experiencing respiratory symptoms: call a POISON CENTER or doctor/physician. **P304+P340** IF INHALED: remove person to fresh air and keep comfortable for breathing. **P305+P351+P338** IF IN EYES: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. **P310** Immediately call a POISON CENTER / doctor. **P370+P378** In case of fire: use water spray, CO_2, foam, powder; fight larger fires with spray or alcohol resistant foam to extinguish.