EXTRACTME®
DNA CLEAN-UP & GEL-OUT KIT

Cat. No. EM26
Version: 1.2018

Kit for total DNA purification and isolation from agarose gels

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I. INTENDED USE

The ***EXTRACTME*** **DNA CLEAN-UP & GEL-OUT KIT** is designed for a rapid and efficient purification of DNA fragments after enzymatic reactions and directly from agarose gels (standard and low-melting point agarose gels run in either a TAE or TBE buffer). It efficiently removes nucleases, enzyme inhibitors, detergents, restriction enzymes, polymerases, divalent ions, agarose, ethidium bromide and other contaminants. The purified DNA can be used in common downstream applications. The kit enables the purification of DNA fragments from 50 bp to 30 kb, as well as plasmid and genomic DNA. However purification of fragments smaller than 100 bp and larger than 10 kb will result in decreased recovery rates. The purification protocol and buffer formulations were optimized for high yields and purity of DNA. 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	EM26-010	EM26-050	EM26-250	
CB Buffer (Clean-Up Binding Buffer)	5 ml	25 ml	125 ml	RT
GB Buffer (Gel-Out Binding Buffer)	5 ml	25 ml	125 ml	RT (in dark)
Wash Buffer (conc.)*	3 ml	15 ml	75 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

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

* Before the first use, add appropriate amount of **96–100% ethanol** to **Wash Buffer** (see the instructions on the bottle label and in the table below). It is recommended to mark the bottle after adding alcohol.

NUMBER OF ISOLATIONS	10 ISOLATIONS	50 ISOLATIONS	250 ISOLATIONS
Catalogue number	EM26-010	EM26-050	EM26-250
Wash Buffer	3 ml	15 ml	75 ml
96–100% ethanol	12 ml	60 ml	300 ml
Total volume	15 ml	75 ml	375 ml

In order to avoid evaporation, ensure that the buffer bottles are tightly closed before storing.

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

- 96–100% PFA ethanol
- sterile microcentrifuge tubes (1.5–2 ml)
- automatic pipettes and sterile DNase-free tips
- disposable gloves
- microcentrifuge with rotor for 1.5–2 ml (≥ 11 000 x g)
- 3 M sodium acetate, pH 5.2 (might be required)
- sterile scalpel or razor
- transilluminator

IV. PRINCIPLE

DNA purification procedure utilizes spin minicolumns with membranes which efficiently and selectively bind nucleic acids. In the first step of the clean-up protocol CB Buffer is added to a DNA sample. It causes proteins to degrade and enables DNA binding to the column membrane while in the gel-out protocol DNA fragments is excised from an agarose gel and incubated in GB Buffer, which enables gel fragment solubilization and protein degradation. As an added convenience, the binding buffers contain a color indicator, which facilitates easy monitoring of the solution's pH for optimal DNA binding. The two-step washing stage efficiently removes impurities and enzyme inhibitors. Purified DNA is eluted with the use either a low ionic strength buffer (Elution Buffer) or water (pH 7.0–9.0) and can be used directly in all downstream applications such as PCR, qPCR, Southern blotting, DNA sequencing, enzymatic restriction, ligation and so forth or stored until ready to use.

V. QUALITY CONTROL

The quality of each production batch (LOT) of the ***EXTRACT****ME* **DNA CLEAN-UP & GEL-OUT KIT** is tested using standard QC procedures. Purified DNA concentration and quality are evaluated by gel electrophoresis and spectrophotometer.

VI. PRODUCT SPECIFICATIONS

SAMPLE MATERIAL

CLEAN-UP: up to 100ul of a DNA sample
GEL-OUT: agarose fragment of up to 300 mg containing DNA

YIELD

Depending on DNA fragment length (in the range of 100 bp – 10 kb):
CLEAN-UP: 90–99%
GEL-OUT: 70–95%

DNA FRAGMENT LENGTH

100 bp – 10 kb
DNA fragments in the 50–100 bp and 10–30 kbp range can also be purified, as can genomic and plasmid DNA, however the efficiency will be decreased.

BINDING CAPACITY

Approx. 25 µg DNA

TIME REQUIRED

5–10 min for clean-up procedure
16–20 min for gel-out procedure

DNA PURITY

A_{260}/A_{280} ratio = 1.7–1.9

VII. SAFETY PRECAUTIONS

- → The use of sterile pipette filter tips is recommended.
- → Avoid cross-contamination of DNA between minicolumns.
- → 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.
- → If ethidium bromide or other harmful chemical components is used for gel electrophoresis image visualization, then suitable protective clothing and disposable nitrile gloves must be worn at all times.
- → While excising the agarose fragment, compliance with all the safety requirements for working with UV light (protective clothing, safety goggles, nitrile disposable gloves) is essential.

VIII. RECOMMENDATIONS AND IMPORTANT NOTES

DNA elution

An optimal volume of Elution Buffer used should be chosen in accordance with the amount of DNA in the sample and with final DNA concentration expected. The use of 30–100 µl of Elution Buffer is recommended.

If high DNA concentration is desired elution's volume may be reduced down to 20 µl. It should be noted that this may reduce the efficiency of DNA retrieval. It is essential to apply Elution Buffer precisely onto the centre of the membrane. In order to maximize the DNA retrieval heat Elution Buffer to 80°C and incubate it on the membrane for 10 minutes.

If full DNA retrieval is required, a second elution should be performed. For second elution, repeat steps 10–13 of the Isolation Protocol (section XI), placing the purification column in a new, sterile 1.5 ml Eppendorf tube.

Elution Buffer

Elution Buffer does not contain EDTA, which may interfere with some enzymatic reactions.

pH monitoring

CB Buffer and GB Buffer contain an indicator, which enables pH monitoring. Yellow indicates that the solution's pH is lower than 7.5, which guarantees optimal DNA binding with the membrane. When the pH is higher than 7.5, solution turns pink. It usually happens when the pH of a DNA sample considerably differs from the standard parameters of the DNA treatment operations (pH > 9.0, when the running buffer for electrophoresis has been used several times or was incorrectly prepared). In such cases, it is essential to add **10 µl 3 M sodium acetate (pH 5.2)**. It will lower the pH, enabling the solution to bind efficiently to the minicolumn membrane.

IX. SAMPLE PREPARATION

CLEAN-UP purification

Transfer the appropriate amount of a DNA sample (no more than 100 µl) to a sterile, 1.5–2 ml Eppendorf tube. Prior to the purification process, DNA samples may be stored at +4°C under DNase-free conditions for a short time or frozen (-80°C is strongly recommended) for a longer period. Avoid repeated freeze/thaw cycles of DNA samples.

GEL-OUT purification

1. Conduct gel electrophoresis using standard or low melting point agarose in either a TAE or TBE buffer until DNA fragments are sufficiently separated. Using high voltage is not recommended, since this may cause an increase in buffer temperature and DNA degradation. Use freshly prepared run buffer and the buffer used for the gel preparation.
2. Weigh a sterile, 1.5–2 ml Eppendorf tube.
3. Excise DNA fragment from the agarose gel using a clean, sharp scalpel or razor blade. Minimize the size of the gel slice by removing any excess agarose (the weight of the agarose slice should not exceed 300 mg). The blade and transilluminator should be cleaned with a DNA remover prior to excision. As far as possible, manipulations should be carried out so as to minimize UV exposure to a few seconds. This is particularly vital when DNA isolated is to be used for sequencing or cloning.
4. Transfer the gel slice into a pre-weighed, sterile, 1.5–2 ml Eppendorf tube and weigh it. If the gel fragment mass exceeds 300 mg, divide it into smaller fragments and transfer them to the other 1.5–2 ml tube.
5. Before starting the isolation process, the gel slice containing DNA fragment may be stored at +4°C or -20°C for up to 1 week under DNase-free conditions.

X. PRIOR TO ISOLATION

1. Mix well each buffer supplied with the kit.
2. Ensure that ethanol has been added to **Wash Buffer**. If not, add appropriate amount of **96–100% ethanol** (the volumes can be found on bottles' labels or in the table given in section II).
3. Examine all buffers. If a sediment occurred in any of them, incubate it at **37°C**, mixing occasionally until the sediment has dissolved. Cool to room temperature.
4. Heat a dry block heater or a water bath to **50°C** – only gel-out purification.
5. Unless otherwise stated, conduct all isolation steps at room temperature.

XI. CLEAN-UP ISOLATION PROTOCOL

1. **Add 5 volumes** of **CB Buffer** to 1 volume of a **DNA sample** (for example add 250 µl CB Buffer to a 50 µl PCR reaction) and vortex for **3 s**.
   - ⚠ For sample preparation method, see instructions given in section IX. Sample preparation.
   - ⚠ The solution should be yellow. If it turns pink after mixing, add 10 µl of 3 M sodium acetate, pH 5.2, and mix thoroughly (see section VIII. Recommendations and Important Notes).
2. Centrifuge the tube briefly in order to recover any remaining liquid from the lid and transfer the whole volume of the mixture into a DNA Purification Column placed in a Collection Tube. Centrifuge for **60 s** at 11 000–15 000 x g.
3. Transfer DNA Purification Column to a new Collection Tube (2 ml).
4. Add **600 µl Wash Buffer** and centrifuge for **30 s** at 11 000–15 000 x g.
5. Discard the filtrate and reuse the Collection Tube.
6. Add **400 µl Wash Buffer** and centrifuge for **30 s** at 11 000–15 000 x g.
7. Discard the filtrate and reuse the Collection Tube.

8. Centrifuge for **60–120 s** at 15 000–21 000 x g.
   ⚠ Wash Buffer contains alcohol, which may interfere with some enzymatic reactions and also decrease the elution efficiency. It is therefore vital to remove the alcohol completely from the minicolumn before elution.

9. Discard Collection Tube and the filtrate and carefully transfer the purification minicolumn to a sterile 1.5 ml Eppendorf microcentrifuge tube.

10. Add **50–100 µl Elution Buffer**, directly onto DNA Purification Column membrane.
    ⚠ Other buffer volumes in the 20–200 µl range may be used. For instructions, see section VIII. Recommendations and important notes.

11. Incubate DNA Purification Column at room temperature for **120 s**.

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

13. Remove DNA Purification Column. Isolated DNA should be stored at **+4°C** or **-20°C** depending on further applications.

XII. GEL-OUT ISOLATION PROTOCOL

1. Excise a gel slice containing the DNA fragment and place it in a 1.5–2 ml Eppendorf tube.
   ⚠ The gel slice mass should not exceed 300 mg. For instructions, see section IX. Sample preparation.

2. Add **500 µl GB Buffer** and mix well by inverting the tube for several times.

3. Incubate the mixture at **50°C** for **5–10 minutes** or until the agarose has completely dissolved. During the incubation, mix the sample by inverting the tube several times.
   ⚠ Ensure that the agarose is completely dissolved before moving on to the next step.
   ⚠ The solution should be yellow. If it turns pink after mixing, add 10 µl of 3 M sodium acetate, pH of 5.2, and mix thoroughly (see section VIII. Recommendations and Important Notes).

4. Centrifuge the tube briefly in order to recover any remaining liquid from the lid and transfer **800 µl** of the **mixture** into a DNA Purification Column placed in a Collection Tube. Centrifuge for **60 s** at 11 000–15 000 x g.
   ⚠ If the volume of the mixture exceeds 800 µl in total, discard the filtrate after centrifugation, then reuse Collection Tube and transfer the remaining mixture into the same minicolumn.

5. Transfer DNA Purification Column to a new Collection Tube (2 ml).

6. Add **700 µl Wash Buffer** and centrifuge for **30 s** at 11 000–15 000 x g.

7. Discard the filtrate and reuse Collection Tube.

8. Repeat steps 6 and 7.

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

   ⚠ Wash buffer contains alcohol, which may interfere with some enzymatic reactions and also decrease the elution efficiency. It is therefore vital to remove the alcohol completely from minicolumn before elution.

10. Discard Collection Tube and filtrate and carefully transfer DNA Purification Column to a sterile, 1.5 ml Eppendorf microcentrifuge tube.

11. Add **50 µl Elution Buffer**, directly onto the purification minicolumn membrane.

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

12. Incubate DNA Purification Column at room temperature for **120 s**.

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

14. Remove DNA Purification Column. Isolated DNA should be stored at **+4°C** or **-20°C** depending on further applications.

XIII. TROUBLESHOOTING

Problem	Possible cause	Solution
Low yield of purified DNA	Ineffective DNA binding to membrane.	Ensure the mixture is yellow after adding CB and GB Buffers. If the color turns pink, add 10 µl of 3 M sodium acetate, pH 5.2.
	Incomplete DNA elution from membrane.	Before applying Elution Buffer to the 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.
	Ethanol was not added to Wash Buffer.	Ensure that 96-100% ethanol was added to Wash Buffer before use.
	Incomplete agarose slice dissolution.	Extend incubation at 50°C until agarose slice is completely lysed. After lysis, incubate sample for an additional 5 minutes.
Column becomes clogged during purification	Incomplete agarose slice dissolution.	Extend incubation at 50°C until agarose slice is completely lysed. After lysis, incubate sample for an additional 5 minutes.
DNA flows out of the lanes in agarose gel	Purified DNA contains residual alcohol.	Repeat the isolation, paying a particular attention to whether any residual Wash Buffer is left in purification column after centrifugation in step 8 in clean-up protocol and step 9 in the gel-out protocol.
Blurred bands in gel electrophoresis image	Running buffer contains nucleases.	Always use freshly prepared buffer for both electrophoresis run and gel preparation.
		Store the gel fragment at +4°C, under DNase-free conditions, for no more than a few days.
	Elution solution contains DNases.	Use fresh elution solution. If water is used instead of Elution Buffer, ensure that it is DNase-free.
Inhibition of downstream enzymatic reactions	Running buffer for electrophoresis was contaminated.	Always use freshly prepared buffer for both the electrophoresis run and gel preparation.
	Purified DNA contains residual salts.	Perform all centrifugation steps at room temperature. Ensure that there is no sediment in Wash Buffer before use.
	Purified DNA contains residual alcohol.	Repeat isolation, paying a particular attention to whether any residual Wash Buffer is left in purification column after centrifugation in step 8 in clean-up protocol and step 9 in gel-out protocol.

Incorrect DNA sequencing results.	Running buffer for electrophoresis was contaminated.	Always use freshly prepared buffer for both electrophoresis run and gel preparation.
	Extensive exposure to UV light.	Minimize the DNA's exposure time to UV light during the excision from gel procedure.
	Equipment has been contaminated.	Clean scalpel or razor blade and transilluminator surface prior to gel slice excision.

XIV. SAFETY INFORMATION

GB Buffer

Warning
H302, H312, H332, H412
P273, P301+P312 P330, P304+P340 P312, EUH032

CB buffer

Danger
H225, H302, H315, H319, H336
P264, P280, P301+P312 P330, P210

EUH032 Contact with acids liberates very toxic gas. **H225** Highly flammable liquid and vapour. **H302** Harmful if swallowed. **H312** Harmful in contact with skin. **H315** Causes skin irritation. **H319** Causes serious eye irritation. **H332** Harmful if inhaled. **H336** May cause drowsiness or dizziness. **H412** Harmful to aquatic life with long-lasting effects. **P264** Wash hands thoroughly after handling. **P273** Avoid release to the environment. **P280** Wear protective gloves/protective clothing/eye protection/face protection. **P301+P312 P330** IF SWALLOWED: Call a POISON CENTER / doctor if you feel unwell. Rinse mouth. **P304+P340 P312** IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER / doctor if you feel unwell. **P210** Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.