EXTRACTME®
DNA GEL-OUT KIT

Cat. No. EM08.1
Version: 1.2019
NEW VERSION

Kit for total DNA isolation from agarose gels

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I. INTENDED USE

The ***EXTRACTME*** **DNA GEL-OUT KIT** is designed for rapid and efficient purification of DNA fragments directly from agarose gels (standard and low-melting point agarose gels run in either a TAE or TBE buffer). Agarose, ethidium bromide and other contaminants from a sample are effectively removed in the purification process. The kit enables the purification of DNA fragments from 50 bp to 20 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 purified DNA can be used in common downstream applications. The purification protocol and buffer formulations were optimized for high yields 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	EM08.1-010	EM08.1-050	EM08.1-250	
GB Buffer (Binding Buffer)	4 ml	20 ml	100 ml	RT
GW Buffer (conc.)* (Wash Buffer)	3.5 ml	16 ml	80 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
Loading Buffer	1 pc	1 pc	1 pc	RT

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

* Prior to the first use, add an appropriate amount of **96-100% ethanol** to **CW Buffer** (see the instructions on the bottle label and in the table below). It is recommended to mark the bottle containing added alcohol.

NUMBER OF ISOLATIONS	10 ISOLATIONS	50 ISOLATIONS	250 ISOLATIONS
Catalogue number	EM08.1-010	EM08.1-050	EM08.1-250
GW Buffer	3.5 ml	16 ml	80 ml
96–100% ethanol	14 ml	64 ml	320 ml
Total volume	17.5 ml	80 ml	400 ml

⚠ **Protect GB Buffer from the sunlight**.

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% ethanol PFA
- 2-propanol
- sterile scalpel or razor
- 1.5–2 ml sterile microcentrifuge tube
- automatic pipettes and pipette tips
- personal protection equipment (lab coat and gloves)
- transilluminator
- microcentrifuge with rotor for 1.5–2 ml (≥ 11 000 x g)
- dry block heater or water bath (up to 50°C)
- 3M sodium acetate, pH 5.2 (might be required)

IV. PRINCIPLE

The DNA purification procedure utilizes spin minicolumns with membranes which efficiently and selectively bind nucleic acids. In the first step, the DNA fragment is excised from an agarose gel and incubated in GB Buffer, which enables gel fragment solubilization and protein degradation. As an added convenience, binding buffer contains a colour 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. The purified DNA is eluted using 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 ***EXTRACTME*** **DNA 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
Agarose fragment of up to 300 mg containing DNA
YIELD
70-95%, depending on DNA fragment length (in the range of 100 bp – 10 kb)
DNA FRAGMENT LENGTH
50 bp – 20 kb Genomic and plasmid DNA, however, the efficiency will be decreased.
BINDING CAPACITY
Approx. 40 µg DNA
TIME REQUIRED
16-20 minutes
DNA PURITY
A_{260}/A_{280} ratio = 1.7 – 1.9

VII. SAFETY PRECAUTIONS

- 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.
- The use of sterile pipette filter tips is recommended.
- Avoid cross-transferral of DNA between minicolumns.
- Guanidine salts residues may form highly reactive compounds when combined with oxidation components. In case of spillage, clean the surface with a detergent water solution.

VIII. RECOMMENDATIONS AND IMPORTANT NOTES

DNA elution

Optimal volume of Elution Buffer used should be chosen in line with the type of DNA in a sample and final DNA concentration expected. The use of 50–100 µl of Elution Buffer is recommended.

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 the membrane.

In order to maximize DNA retrieval, heat Elution Buffer to 70°C and incubate it on the membrane for 10 minutes.

If full DNA retrieval is required 200 µl Elution Buffer should be used. However it will result in DNA dilution. Second elution can be also be performed. For second elution, repeat steps 11–14 of the Isolation Protocol (section XI), placing purification column in a new, sterile 1.5 ml Eppendorf tube.

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

pH monitoring

GB Buffer contains an indicator, which enables pH monitoring. Yellow indicates that the solution's pH is lower than 7.0 and guarantees an optimal DNA binding with the membrane. When the pH is higher than 7.0, solution turns pink. It usually happens when the pH of DNA sample considerably differs from the standard parameters of DNA treatment operations (pH>9.0). In this case, it is essential to add 10 µl of 3 M sodium acetate (pH 5.2). It will lower the pH, enabling the solution to bind efficiently with the minicolumn membrane.

Loading Buffer

Loading Buffer is provided for analysis of purified DNA samples with the use of gel electrophoresis. Loading Buffer contains 3 dyes (bromophenol blue, xylene cyanol and orange G). Loading Buffer is concentrated by a factor of six, thus, in order to obtain the most satisfying results mix 2 µl of Loading Buffer with 10 µl of purified DNA.

IX. SAMPLE PREPARATION

1. Conduct gel electrophoresis using standard or low melting point agarose in either a TAE or TBE buffer until the 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 buffer used for gel preparation.
2. Weigh a sterile, 1.5–2 ml Eppendorf tube.
3. Excise DNA fragment from agarose gel using a clean, sharp scalpel or razor blade. Minimize size of gel slice by removing any excess agarose (weight of agarose slice should not exceed 300 mg). 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 gel slice into a pre-weighed, sterile, 1.5–2 ml Eppendorf tube and weigh it. If gel fragment mass exceeds 300 mg, divide it into smaller fragments and transfer them to other 1.5–2 ml tube.
5. Before starting isolation process, 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 kit.
2. Ensure that ethanol has been added to **GW Buffer**. If not, add appropriate amount of **96–100% ethanol** (volumes can be found on bottles' labels or in table given in section II).
3. Examine all buffers. If a sediment occurred in any of them, incubate it at 37°C (**GW Buffer**) or at 50–60°C (other buffers) mixing occasionally until sediment has dissolved. Cool to room temperature.
4. Heat a dry block heater or a water bath to 50°C.

XI. ISOLATION PROTOCOL

Ensure that ethanol has been added to GW Buffer (section II).

STEP 1

Excise a gel slice containing DNA fragment and place it in a 1.5–2 ml Eppendorf tube.

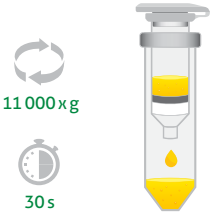
⚠ The gel slice mass should not exceed 300 mg. For instructions, see section IX. Sample preparation.

Add **400 µl GB Buffer** and mix well by inverting tube for several times.

Incubate the mixture at **50°C** for **5–10 minutes** or until the agarose has completely dissolved. During incubation, mix the sample by inverting tube several times.

⚠ Ensure that agarose is completely dissolved before moving on to the next step.

STEP 2



Add **250 µl isopropanol** (not included in kit) and mix well by inverting tube several times.

Centrifuge a tube briefly in order to recover any remaining liquid from the lid and transfer maximum **800 µl** of the **mixture** into a DNA purification minicolumn placed in a collection tube. Centrifuge for **30 s** at **11 000 x g**. Discard the filtrate.

⚠ If volume of mixture exceeds 800 µl in total, discard filtrate after centrifugation, then reuse collection tube and transfer remaining mixture into the same minicolumn.

Transfer purification minicolumn to a new collection tube (2 ml).

STEP 3

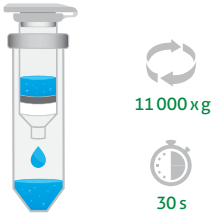
Add **700 µl GW Buffer** and centrifuge for **30 s** at **11 000 x g**.

Discard the filtrate and reuse the collection tube.

Recommended: repeat previous washing step.

Add **700 µl GW Buffer** and centrifuge for **30 s** at **11 000 x g**.

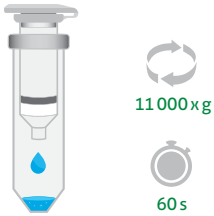
Discard the filtrate and reuse the collection tube.



STEP 4

Centrifuge for **60 s** at **11 000 x g**. Discard the collection tube and the filtrate.

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



STEP 5

Carefully transfer the purification minicolumn to a sterile, 1.5 ml Eppendorf microcentrifuge tube.

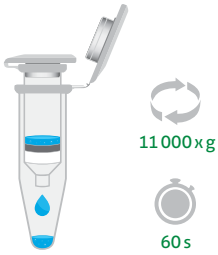
Add **50 µl Elution Buffer**, directly onto the purification minicolumn membrane.

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

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

Centrifuge for **60 s** at **11 000 x g**.

Remove the minicolumn. Isolated DNA should be stored at +4°C or -20°C depending on further applications.



XII. TROUBLESHOOTING

Problem	Possible cause	Solution
Low yield of purified DNA.	Incomplete agarose slice dissolution.	Extend incubation at 50°C until agarose slice is completely dissolved. Then incubate sample for an additional 5 minutes.
	Ineffective DNA binding to a membrane.	After GB Buffer has been added, ensure that mixture is yellow. If it turns pink, add 10 µl of 3 M sodium acetate, pH of 5.2.
	Ethanol was not added to wash buffer.	Ensure that 96–100% ethanol was added to GW Buffer before use.
	Incomplete DNA elution from membrane.	Before applying Elution Buffer to the membrane, heat it to 70°C. Apply Elution Buffer directly to centre of membrane. Extend incubation time with Elution Buffer to 10 min. Perform second elution.
	pH of water used for elution is lower than 7.0.	Use Elution Buffer for DNA elution.
Column becomes clogged during purification.	Incomplete agarose slice dissolution.	Extend incubation at 50°C until agarose slice is completely dissolved. Then incubate sample for an additional 5 minutes.
DNA flows out of lanes in the agarose gel.	Purified DNA contains residual ethanol.	Repeat isolation, giving particular attention to ensuring that no residual GW Buffer is left in purification minicolumn after centrifugation in step 9.
Blurred bands in gel electrophoresis image.	Running buffer contains nucleases.	Always use freshly prepared buffer for both electrophoresis run and gel preparation.
		Store 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 electrophoresis run and gel preparation.
	Purified DNA contains residual salts.	Perform all centrifugation steps at room temperature. Ensure that there is no sediment in GW Buffer before use.
	Purified DNA contains residual ethanol.	Repeat isolation, giving particular attention to ensuring that no residual GW Buffer is left in purification minicolumn after centrifugation in step 9.

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 the UV light.	Minimize DNA's exposure time to UV light during excision from gel procedure.
	Equipment has been contaminated.	Clean scalpel or razor blade and transilluminator surface prior to gel slice excision.

XIII. SAFETY INFORMATION

GB Buffer

Warning

H302, H312, H332, H412

P261, P264, P270, P271, P273, P280, P301+P312 P330, P302+P352 P312, P304+P340 P312, P363, EUH032

H302 Harmful if swallowed. **H312** Harmful in contact with skin. **H332** Harmful if inhaled. **H412** Harmful to aquatic life with long-lasting effects. **P261** Avoid breathing dust/fumes/gas/mist/vapours/spray. **P264** Wash hands thoroughly after handling. **P270** Do not eat, drink or smoke when using this product. **P271** Use only outdoors or in a well-ventilated area. **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. **P302+P352 P312** IF ON SKIN: Wash with plenty of water. Call a POISON CENTER / doctor if you feel unwell. **P363** Wash contaminated clothing before reuse. **P304+P340 P312** IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER / doctor if you feel unwell. **EUH032** Contact with acids liberates very toxic gas.