EXTRACTME®
DNA CLEAN-UP KIT

Cat. No. EM07
Version: 1.2017

Kit for DNA purification after enzymatic reactions

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I. INTENDED USE

The ***EXTRACTME*** **DNA CLEAN-UP KIT** is designed for the rapid and efficient purification of DNA fragments after enzymatic reactions. It efficiently removes nucleases, enzyme inhibitors, detergents, restriction enzymes, polymerases, divalent ions, salts etc. 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	150 ISOLATIONS	250 ISOLATIONS	3 ISOLATIONS (DEMO)
Catalogue number	EM07-010	EM07-050	EM07-150	EM07-250	EM07-D
CB Buffer (Binding Buffer)	2.5 ml	25 ml	75 ml	125 ml	1.5 ml
CW Buffer (conc.)* (Wash Buffer)	2.2 ml	11 ml	33 ml	55 ml	3 ml**
Elution Buffer	2 ml	10 ml	3x 10 ml	5x 10 ml	600 µl
DNA Purification Columns	10 pcs	50 pcs	3x 50 pcs	5x 50 pcs	3 pcs
Collection Tubes (2 ml)	10 pcs	50 pcs	3x 50 pcs	5x 50 pcs	3 pcs

NUMBER OF ISOLATIONS	10 ISOLATIONS	50 ISOLATIONS	150 ISOLATIONS	250 ISOLATIONS
Catalogue number	EM07-010	EM07-050	EM07-150	EM07-250
CW Buffer	2.2 ml	11 ml	33 ml	55 ml
96-100% ethanol	8.8 ml	44 ml	132 ml	220 ml
Total volume	11 ml	55 ml	165 ml	275 ml

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

⚠ **N.B.: the **CW Buffer** in **DEMO** kit (cat no. EM07-D) already contains ethanol.

All kit components should be stored at room temperature (15-20˚C). In order to avoid evaporation, ensure that the buffer bottles are tightly closed before storing. Under proper storage conditions, the kit will remain stable for at least 12 months.

III. ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

- → 96-100% ethanol PFA
- → 1.5-2 ml sterile microcentrifuge tubes
- → automatic pipettes and pipette tips
- → disposable gloves
- → microcentrifuge with rotor for 1.5-2 ml (>11k x g)
- → dry block heater or water bath (up to 70˚C)
- → 3 M 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 CB Buffer is added to a DNA sample. It causes proteins to degrade and enables DNA binding to the column membrane. As an added convenience, the 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 CLEAN-UP** kit is tested using standard QC procedures. The purified DNA concentration, quality and stability are evaluated by gel electrophoresis and spectrophotometer.

VI. PRODUCT SPECIFICATIONS

SAMPLE MATERIAL
up to 100 µl of a DNA sample
YIELD
90-99%, depending on DNA fragment length (in the range of 100 bp – 10 kb)
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 minutes
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-transferral of DNA between minicolumns.
- Guanidine salts residues may form highly reactive compounds when combined with oxidation compounds. In case of spillage, clean the surface with a detergent water solution.

VIII. RECOMMENDATIONS AND IMPORTANT NOTES

DNA elution

The optimal volume of the elution buffer used should be chosen in line with the amount of DNA in the sample and to the final DNA concentration expected. The use of 30-100 µl of the Elution Buffer is recommended.

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

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

pH monitoring

The CB Buffer contains an indicator, which enables pH monitoring. Yellow indicates that the pH of the solution is lower than 7.5, which guarantees optimal DNA binding to the membrane. When the pH is higher than 7.5, solution will turn pink. It may happen on the occasion, when the pH of a DNA sample considerably differs from the standard parameters of the DNA treatment operations (pH>9.0). In this case, 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

Transfer the appropriate amount of a DNA sample (no more than 100 µl) to a sterile, 1.5-2 ml Eppendorf tube. Before starting the purification process, the 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 time. Avoid subjecting the DNA sample to repeated freeze/thaw cycles.

X. BEFORE STARTING

1. Mix well each buffer supplied with the kit.
2. Ensure that ethanol has been added to the **CW Buffer**. If not, add appropriate amount of 96-100% ethanol (the volumes can be found on bottle labels or in the table given in section II).
3. Examine the buffers. If a sediment occurred in any of them, incubate the bottle with the solution at 37˚C, mixing occasionally until the sediment has dissolved. Cool to room temperature.
4. Heat a sufficient amount of the **Elution Buffer** to 70˚C.
5. Unless otherwise stated, conduct all isolation steps at room temperature.

XI. ISOLATION PROTOCOL

1. **Add 5 volumes** of the **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 minicolumn placed in a collection tube. Centrifuge for 1 min at 11-15k x g.
3. Transfer the purification minicolumn to a new collection tube (2 ml).
4. Add **600 µl CW Buffer** and centrifuge for 30 s at 11-15k x g.
5. Discard the filtrate and reuse the collection tube.
6. Add **400 µl CW Buffer** and centrifuge for 30 s at 11-15k x g.
7. Discard the flow-through and reuse the collection tube.

8. Centrifuge for 1-2 min at 15-21k x g.

   ⚠ The 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 the collection tube and the flow-through and carefully transfer the purification minicolumn to a sterile 1.5 ml Eppendorf microcentrifuge tube.

10. Add **50-100 µl Elution Buffer**, pre-heated to 70°C 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.

11. Incubate the minicolumn at room temperature for 2 min.

12. Centrifuge at 11-15k x g for 1 min.

13. Remove the minicolumn. The isolated DNA is ready for use in downstream applications or for short-term storage at +4°C or for long-term storage at -20°C.

XII. TROUBLESHOOTING

Problem	Possible cause	Solution
Low yield of purified DNA.	Ineffective DNA binding to the membrane.	Ensure the mixture is yellow after adding the CB Buffer. If the colour turns pink, add 10 µl of 3 M sodium acetate, pH 5.2.
	Incomplete DNA elution from the membrane.	Before applying the Elution Buffer to the membrane, heat it to 80°C. Apply the Elution Buffer directly to the centre of the membrane. Extend the incubation time with the Elution Buffer to 10 min. Perform second elution. Increase volume of the Elution Buffer to 200 µl.
	The pH of the water used for elution is lower than 7.0.	Use Elution Buffer for DNA elution.
	Ethanol was not added to the wash buffer.	Ensure that 96-100% ethanol was added to the CW Buffer before use.
DNA flows out of the lanes in the agarose gel.	The purified DNA contains residual alcohol.	Repeat the isolation, giving particular attention to ensuring that no residual CW Buffer is left in the purification column after centrifugation in step 8.
Blurred bands in the gel electrophoresis image.	The elution solution contains DNases.	Use fresh elution solution. If water is used instead of the Elution Buffer, ensure that it is DNase-free.
Inhibition of downstream enzymatic reactions.	The purified DNA contains residual salts.	Perform all centrifugation steps at room temperature. Ensure that there is no sediment in the CW Buffer before use.
	The purified DNA contains residual alcohol.	Repeat the isolation, giving particular attention to ensuring that no residual CW Buffer is left in the purification column after centrifugation in step 8.

XIII. SAFETY INFORMATION

CB Buffer

Hazard
H225, H302, H315, H319, H336
P305+P351+P338, P302+P352, P210, P233

H225 Highly flammable liquid and vapour. **H302** Harmful if swallowed. **H315** Causes skin irritation. **H319** Causes serious eye irritation. **H336** May cause drowsiness or dizziness. **P210** Keep away from heat/sparks/open flames/hot surfaces. No smoking. **P305 + P351 + P338 IF IN EYES:** Rinse continuously with water for several minutes. Remove contact lenses if present and easy to do – continue rinsing. **P302+P352** IF ON SKIN: Wash with plenty of soap and water. **P233** Keep container tightly closed.