EXTRACTME®
DNA CLEAN-UP KIT

Cat. No. EM07.1
Version: 1.2018
NEW VERSION

Kit for DNA purification after enzymatic reactions

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I. INTENDED USE

The ***EXTRACTME*** **DNA CLEAN-UP KIT** is designed for a rapid and efficient purification of DNA fragments after enzymatic reactions. It efficiently removes nucleases, primers, enzyme inhibitors, detergents, restriction enzymes, polymerases, divalent ions, salts, mineral oil, etc. Purified DNA may be used in common downstream applications. Primers from PCR reactions are eliminated quantitatively while small DNA fragments are still bound and purified with high recovery. The kit enables purification of DNA fragments from 50 bp to 20 kbp, as well as plasmid and genomic DNA. 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*
Catalogue number	EM07.1-010	EM07.1-050	EM07.1-250	
CB Buffer (Binding Buffer)	4 ml	20 ml	100 ml	RT
CW 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

*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	EM07.1-010	EM07.1-050	EM07.1-250
CW 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

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

Expiry date

Under proper conditions the product can be stored until the expiry date (the information on the label) or at least for 12 months after first opening.

III. ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

- 96-100% ethanol PFA
- 1.5-2 ml sterile microcentrifuge tubes
- automatic pipettes and pipette tips
- personal protecion equipment (lab coat and gloves)
- microcentrifuge with rotor for 1.5-2 ml (≥ 11 000 x g)
- vortex mixer

IV. PRINCIPLE

DNA purification procedure utilizes spin minicolumns with membranes which efficiently and selectively bind nucleic acids. First, CB Buffer is added to DNA sample. It causes proteins to degrade and enables DNA to bind with the column's membrane. The binding buffer contains a color indicator, that facilitates an 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 using either 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, DNA sequencing, enzymatic restriction, DNA ligation, etc. 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 with the use of standard QC procedures. Purified DNA concentration and quality are evaluated by gel electrophoresis and spectrophotometer.

VI. PRODUCT SPECIFICATIONS

SAMPLE MATERIAL

up to 200 µl of DNA sample

YIELD

60-99% – depending on DNA fragment length

DNA FRAGMENT LENGTH

50 bp – ~20 kbp
genomic and plasmid DNA, however the efficiency will be decreased

BINDING CAPACITY

approx. 40 µg DNA

TIME REQUIRED

10 min for 6 PCR purifications

DNA PURITY

$A_{260/280}$ ratio = 1.7-1.9

VII. SAFETY PRECAUTIONS

- → Use of sterile filter tips is recommended.
- → Avoid cross-contamination 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

The optimal volume of Elution Buffer used should be chosen in accordance with the amount of DNA in a sample and to final DNA concentration expected. The use of 15-30 µl of Elution Buffer is recommended.

It is essential to apply Elution Buffer precisely onto the centre of the membrane. In order to maximize DNA recovery, the following modifications should be applied:

→ Heat Elution Buffer up to 70°C, apply onto the column and incubate at room temperature for 5 min.

→ Carry out 2 or 3 elution steps with 15-30 µl Elution Buffer.

To assure complete DNA recovery from the membrane, use 200 µl Elution Buffer.

Elution Buffer

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

pH monitoring

CB 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

Transfer an appropriate amount of DNA sample (no more than 200 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 (-20°C or -80°C) for a longer period. Avoid repeated freeze/thaw cycles of DNA samples.

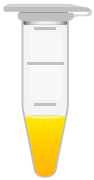
X. PRIOR TO ISOLATION

1. Mix well each buffer supplied with the kit.
2. Ensure that ethanol has been added to **CW Buffer**. If not, add appropriate amount of **96-100% ethanol** (volumes can be found on bottles' labels or in the table given in the 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.

XI. ISOLATION PROTOCOL

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

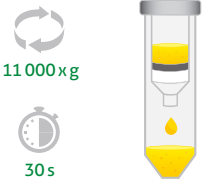
STEP 1



Add 2 volumes of **CB Buffer** to a **1 volume** of **DNA sample** (for example add 100 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.

STEP 2

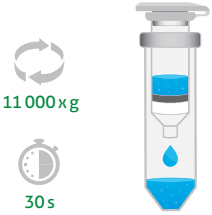


Centrifuge a tube briefly in order to recover any remaining liquid from the lid and transfer the whole volume of the mixture into a DNA purification minicolumn placed in a collection tube (2 ml).

Centrifuge for **30 s** at **11 000 x g**. Discard the filtrate.

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

STEP 3



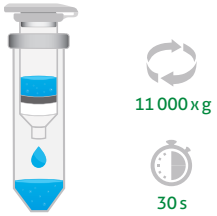
Add **700 µl CW Buffer** and centrifuge for **30 s** at **11 000 x g**.

Discard the filtrate and reuse the collection tube.

Recommended: repeat previous washing step.

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

Discard the filtrate and reuse the collection tube.

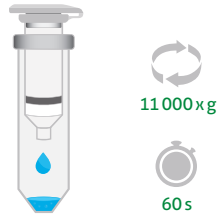


STEP 4

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

Discard the collection tube and the filtrate.

⚠ CW 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 the minicolumn before elution.



STEP 5

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

Add **15-30 µl Elution Buffer**, directly onto the purification minicolumn membrane.

Incubate the minicolumn at room temperature for 60 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.

XI. TROUBLESHOOTING

Problem	Possible cause	Solution
Low yield of purified DNA.	Ineffective DNA binding with the membrane.	Ensure the mixture is yellow after adding CB Buffer. If the color turns pink, add 10 µl of 3 M sodium acetate, pH 5.2.
	Incomplete DNA elution from the membrane.	Before applying Elution Buffer to the membrane, heat it to 70°C. Apply Elution Buffer directly to the centre of the membrane. Extend incubation time with Elution Buffer to 5 min. Perform a second elution.
	The pH of 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 CW Buffer before use.
DNA flows out of lanes in the agarose gel.	Purified DNA contains residual alcohol.	Repeat the isolation, ensuring that no residual CW Buffer is left in the purification column after centrifugation in step 4.
Blurred bands in the gel electrophoresis image.	The elution solution contains DNases.	Use a fresh elution solution. If water is used instead of Elution Buffer, ensure that it is DNase-free.
Inhibition of downstream enzymatic reactions.	Purified DNA contains residual salts.	Perform all centrifugation steps at room temperature. Ensure that there is no sediment in CW Buffer prior to use.
	Purified DNA contains residual alcohol.	Repeat the isolation, ensuring that no residual CW Buffer is left in the purification column after centrifugation in step 4.

XII. SAFETY INFORMATION

CB Buffer

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

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. **P264** Wash hands thoroughly after handling. **P280** Wear protective gloves/protective clothing/eye protection/face protection. **P301+312 P330** IF SWALLOWED: call a POISON CENTER/doctor/if you feel unwell. Rinse mouth.