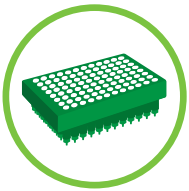
EXTRACTME®
DNA CLEAN-UP 96-WELL KIT

Cat. No. EM22
Version: 1.2017

Kit for total DNA purification after enzymatic reactions in 96-well format



I. INTENDED USE

The ***EXTRACTME*** **DNA CLEAN-UP 96-WELL 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	2x 96-WELL PLATES	10x 96-WELL PLATES
Catalogue number	EM22-192	EM22-960
CB Buffer (Binding Buffer)	96 ml	480 ml
CW Buffer (conc.)* (Wash Buffer)	46 ml	5 x 46 ml
Elution Buffer	39 ml	5 x 39 ml
DNA Binding Plates	2 pcs	10 pcs
Collection Plates	2 pcs	10 pcs
DNA Elution Plates	2 pcs	10 pcs
Elution Adhesive Seals	2 pcs	10 pcs

* 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.

NUMBER OF ISOLATIONS	2x 96-WELL PLATES	10x 96-WELL PLATES
Catalogue number	EM22-192	EM22-960
CW Buffer	46 ml	5 x 46 ml
96-100% ethanol	184 ml	5 x 184 ml
Total volume	230 ml	5 x 230 ml

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
- multichannel pipettes and pipette tips
- disposable gloves
- microcentrifuge with rotor for plates (≥3k x g)
- dry block heater or water bath (up to 70°C)
- vortex mixer
- adhesive seals
- vacuum manifold and vacuum pump (producing pressure of -400 to -600 mbar) or automated liquid handling workstations
- 3 M sodium acetate, pH 5.2 (might be required)

IV. PRINCIPLE

The DNA purification procedure utilizes 96-minicolumns plates 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 ***EXTRACT****ME* **DNA CLEAN-UP 96-WELL KIT** is tested using standard QC procedures. The purified DNA concentration, quality and stability are evaluated by gel electrophoresis and spectrophotometer. In addition, the functional quality is tested by qPCR and digestion with restriction enzymes.

VI. PRODUCT SPECIFICATIONS

SAMPLE MATERIAL

Up to 100 µl of a DNA sample per well

YIELD

80-95%, 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. 20 µg DNA

TIME REQUIRED

- approx. 30 minutes for purification using centrifuge
- approx. 25 minutes for purification using vacuum manifold

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 wells.
- 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

Vacuum manifold use

Establish a reliable vacuum source for the ***EXTRACTME*** **DNA CLEAN-UP 96-WELL KIT** vacuum manifold protocol. The manifold may be used with a vacuum pump or water aspirator. Use a vacuum pressure of -400 to -600 mbar or reduce the vacuum pressure until a flow rate 1-2 drops per second is achieved. Using higher vacuum pressure than recommended may cause sample splattering, while using lower vacuum pressure will affect the elution resulting in lower recovery.

Keep all unused well in the DNA Binding Plate sealed with an adhesive seal during purification to obtain a uniform vacuum and avoid contaminating unused wells.

Pressure conversions

	Recommended pressure (mbar)
	-400 to -600
Conversion from millibars	**Multiply by**
Millimeters of Mercury (mmHg)	0.75
Kilopascals (kPa)	0.1
Inches of Mercury (inchHg)	0.0295
Torrs (Torr)	0.75
Atmospheres (atmos)	0.000987
Pounds per Square Inch (psi)	0.0145

To check the vacuum pressure place an unused DNA Binding Plate on top of the vacuum pressure on the vacuum regulator. Adjust the vacuum pressure on the regulator to obtain the recommended pressure.

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 9-12 of the Isolation Protocol while purification using centrifuge (see section XI.I) or steps 17-20 while purification using vacuum manifold (see section XI.II), placing DNA Binding Plate in a new DNA Elution Plate (provided by the user).

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)** per sample. 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 per well) to a sterile, 1.5-2 ml Eppendorf tube or to a well of a 96 deep-well plate. 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

I. Purification using centrifuge:

1. **Add 5 volumes** of the **CB Buffer** to **1 volume** of a **DNA sample** into each well of a 96 deep-well plate (provided by the user; for example add 250 µl CB Buffer to a 50 µl PCR reaction) and mix well.
   - ⚠ 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. Seal the plate with an adhesive seal (provided by the user) and mix well by vortexing for 10 s.
3. Centrifuge a 96 deep-well plate briefly and transfer the whole volume of the mixture into a DNA Binding Plate placed in a Collection Plate (supplied with the kit) using multichannel pippettor.
   - ⚠ Extensive, high speed centrifugation is not recommended since it may cause the DNA precipitation.
4. Centrifuge the stacked plates for 5-10 min at minimum 3k x g. Discard the flow-through and reuse the Collection Plate.
   - ⚠ If not all of the mixture passes through the membrane, repeat the centrifugation for 2-5 min at minimum 3 k x g. Should the problem persist, it means that the material was insufficiently homogenized or the digestion time was too short or too much sample material was used for the isolation.
5. Add **600 µl CW Buffer** into each well of the DNA Binding Plate and centrifuge for 3 min at minimum 3k x g. Discard the flow-through and reuse the Collection Plate.
6. Add **400 µl CW Buffer** into each well of the DNA Binding Plate and centrifuge for 3 min at minimum 3k x g. Discard the flow-through and reuse the Collection Plate.

7. Centrifuge for 20 min at minimum 3k x g or place the DNA Binding Plate in an incubator for 10 min at **70°C** to evaporate residual alcohol.
   - ⚠ 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 DNA Binding Plate before elution.
   - ⚠ To ensure the completely drying of the membrane – do not seal the plate.
   - ⚠ Removal of alcohol by evaporation at 70°C is more efficient than prolonged centrifugation.

8. Discard the Collection Plate and the flow-through and carefully transfer the DNA Binding Plate to the DNA Elution Plate.

9. Add **50-100 µl Elution Buffer**, pre-heated to 70°C directly onto the centre of the membrane in each well.
   - ⚠ Other buffer volumes in the 20-200 µl range may be used. For instructions, see section VIII. Recommendations and important notes.

10. Incubate the DNA Binding Plate at room temperature for 2 min.

11. Centrifuge the stacked plates at 3k x g for 2 min.

12. Remove the DNA Purification Plate then seal tightly DNA Elution Plate with Elution Adhesive Seal. 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.

XI. ISOLATION PROTOCOL

II. Purification using vacuum manifold:

1. **Add 5 volumes** of the **CB Buffer** to **1 volume** of a **DNA sample** into each well of a 96 deep-well plate (provided by the user).
   - ⚠ 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. Seal the plate with an adhesive seal (provided by the user) and mix well by vortexing for 10 s.
3. Centrifuge the 96 deep-well plate briefly.
   - ⚠ Extensive, high speed centrifugation is not recommended since it may cause the DNA precipitation.
4. Prepare the vacuum manifold according to manufacturer's instructions.
   - ⚠ If using an automated liquid handling workstation, prepare the workstation deck as recommended by the manufacturer.
5. Place the DNA Binding Plate on top of the manifold.
6. Transfer the whole volume of the mixture into a DNA Binding Plate using multichannel pippettor.
   - ⚠ Unused wells should be covered with an adhesive seal (provided by the user).
7. Apply vacuum for 1-2 min until mixture passes through the DNA Binding Plate. Release the vacuum.
8. Add **600 µl CW Buffer** into each well of the DNA Binding Plate.
9. Apply vacuum for 2 min. Release the vacuum.
10. Add **400 µl CW Buffer** into each well of the DNA Binding Plate.

11. Apply vacuum for 2 min. Release the vacuum.
12. Place the DNA Binding Plate on a stack of paper towels and gently tap to remove residua liquid from the nozzles. Replace the DNA Binding Plate on the manifold.
13. Apply vacuum for 10 minutes or place the DNA Binding Plate in an incubarot for 10 min at 70°C to evaporate residual alcohol.
    ⚠ Removal of alcohol by evaporation at 70°C is more efficient than prolonged vacuum membrane drying.
14. Disassemble the manifold to remove the waste tray. Discard the flow-through.
15. Assemble the vacuum manifold with the DNA Elution Plate.
16. Place the DNA Binding Plate onto the vacuum manifold.
17. Add **50-100 µl Elution Buffer**, pre-heated to 70°C, directly onto the center of the membrane in each well.
    ⚠ Other buffer volumes in the 20-200 µl range may be used. For instructions, see section VIII. Recommendations and important notes.
18. Incubate the DNA Binding Plate at room temperature for 2 min.
19. Apply vacuum for 2 min. Release the vacuum.
20. Disassemble the vacuum manifold to remove the DNA Elution Plate then seal tightly DNA Elution Plate with Elution Adhesive Seal. The 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
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 per sample.
	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 step 7 (Purification using centrifuge XI.I) or step 13 (Purification using vacuum manifold XI.II).
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 step 7 (Purification using centrifuge XI.I) or step 13 (Purification using vacuum manifold XI.II).

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.