Kit for total DNA purification and isolation from agarose gels in low elution volume
I. INTENDED USE

The **EXTRACTme DNA CLEAN-UP AND GEL-OUT MICRO SPIN KIT** is designed for the 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) with an extremely low elution volume of only 5 µl. 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 35 bp to 30 kb for clean-up procedure and from 100 bp to 30 kbp for gel-out procedure, as well as plasmid and genomic DNA. However purification of fragments smaller than 50 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

<table>
<thead>
<tr>
<th>NUMBER OF ISOLATIONS</th>
<th>10 ISOLATIONS</th>
<th>50 ISOLATIONS</th>
<th>250 ISOLATIONS</th>
<th>3 ISOLATIONS (DEMO)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalogue number</td>
<td>EM28-010</td>
<td>EM28-050</td>
<td>EM28-250</td>
<td>EM28-D</td>
</tr>
<tr>
<td>CB Buffer (Clean-Up Binding Buffer)</td>
<td>6 ml</td>
<td>30 ml</td>
<td>150 ml</td>
<td>1.8 ml</td>
</tr>
<tr>
<td>GB Buffer (Gel-Out Binding Buffer)</td>
<td>5 ml</td>
<td>25 ml</td>
<td>125 ml</td>
<td>1.5 ml</td>
</tr>
<tr>
<td>Wash Buffer (conc.)*</td>
<td>2.2 ml</td>
<td>11 ml</td>
<td>52.5 ml</td>
<td>3.5 ml**</td>
</tr>
<tr>
<td>Elution Buffer</td>
<td>2 ml</td>
<td>10 ml</td>
<td>5x 10 ml</td>
<td>600 µl</td>
</tr>
<tr>
<td>DNA Purification Micro Spin Columns</td>
<td>10 pcs</td>
<td>50 pcs</td>
<td>5x 50 pcs</td>
<td>3 pcs</td>
</tr>
<tr>
<td>Collection Tubes (2 ml)</td>
<td>10 pcs</td>
<td>50 pcs</td>
<td>5x 50 pcs</td>
<td>3 pcs</td>
</tr>
</tbody>
</table>

* Before the first use, add appropriate amount of 96-100% ethanol to the Wash 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 Wash Buffer in DEMO kit (cat no. EM28-D) already contains ethanol.
### 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)
- sterile scalpel or razor
- transilluminator

### IV. PRINCIPLE

The DNA purification procedure utilizes spin minicolumns with membranes which efficiently and selectively bind nucleic acids. In the first step of the clean-up protocol the 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 the DNA fragments is excised from an agarose gel and incubated in the GB Buffer, which enables gel fragment solubilization and protein degradation. As an added convenience, the binding buffers contain 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 AND GEL-OUT MICRO SPIN 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 | CLEAN-UP: DNA sample  
GEL-OUT: agarose fragment of up to 150 mg containing DNA |
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>YIELD</td>
<td>Depending on DNA fragment length (in the range of 100 bp – 10 kb): 70-90 %</td>
</tr>
</tbody>
</table>
| DNA FRAGMENT LENGTH | CLEAN-UP: 100 bp – 10 kb  
DNA fragments in the 35-100 bp and 10-30 kbp range can also be purified, as can genomic and plasmid DNA, however the efficiency will be decreased.  
GEL-OUT: 100 bp – 10 kb  
DNA fragments in the 10-30 kbp range can also be purified, as can genomic and plasmid DNA, however the efficiency will be decreased. |
| BINDING CAPACITY | Approx. 6 µg DNA |
| TIME REQUIRED | approx. 10 min for clean-up procedure  
approx. 15 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-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.
→ If ethidium bromide or other harmful chemical compound 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
The optimal volume of the elution buffer used should be chosen in line with the presumed amount of DNA in the sample and with the final DNA concentration expected.

If a high DNA concentration is desired, the elution volume may be reduced down to 5 µl. It is important to notice that in such case the final eluent volume will be reduced each time for about (1-1.5 µl) giving, in case of 5 µl elution, c.a. 3.5-4 µl as a final max eluent volume. 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 clean-up Isolation Protocol (section XI) and steps 12-15 of the gel-out Isolation Protocol (section XII), placing the purification column in a new, sterile 1.5 ml Eppendorf tube.

In case of large fragments of DNA, gDNA, pDNA or when high DNA concentration is expected, we suggest to increase the initial elution volume up to 10 µl. Alternatively, if needed, the last elution step can be repeated for 2-3 times.

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

pH monitoring
The CB Buffer and GB Buffer contain 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, 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 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.

GEL-OUT purification
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 the buffer used for the gel preparation.
2. Weigh a sterile, 1.5-2 ml Eppendorf tube.
3. Excise the 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 150 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 the 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 150 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 the DNA fragment may be stored at +4°C or -20°C for up to 1 week under DNase-free conditions.
X. BEFORE STARTING

1. Mix well each buffer supplied with the kit.
2. Ensure that ethanol has been added to the Wash 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. Heat a dry block heater or a water bath to 50°C – only gel-out purification.
6. Unless otherwise stated, conduct all isolation steps at room temperature.
XI. CLEAN-UP ISOLATION PROTOCOL

1. **Add 2-10 volumes** of the **CB Buffer** to 1 volume of a **DNA sample** (see table below) and vortex for 3 s.

<table>
<thead>
<tr>
<th>Application</th>
<th>DNA Binding Buffer: Sample</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA fragments 100 bp–10 kbp</td>
<td>5:1</td>
<td>500 μl : 100 μl</td>
</tr>
<tr>
<td>DNA fragments &lt;100 bp</td>
<td>10:1</td>
<td>500 μl : 50 μl</td>
</tr>
<tr>
<td>Plasmid, genomic DNA DNA fragment &gt;10 kbp</td>
<td>2:1</td>
<td>200 μl : 100 μl</td>
</tr>
</tbody>
</table>

⚠️ 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 Micro Spin Column placed in a Collection Tube. **Centrifuge for 1 min at 11-15k x g.**

⚠️ If the volume of the mixture exceeds 800 μl in total, discard the flow-through after centrifugation, then reuse the Collection Tube and transfer the remaining mixture into the same minicolumn.

3. **Transfer the DNA Purification Micro Spin Column to a new Collection Tube.**

4. **Add 500 μl Wash Buffer** and centrifuge for 30 s at 11-15k x g.

5. **Discard the flow-through and reuse the Collection Tube.**
6. Add **500 μl Wash 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 DNA Purification Micro Spin Column to a sterile 1.5 ml Eppendorf microcentrifuge tube.

10. Add **≥5 μl Elution Buffer**, pre-heated to 70°C directly onto the purification minicolumn membrane.

⚠️ It is essential to apply the elution buffer precisely onto the centre of the membrane.

⚠️ Other buffer volumes may be used. For instructions, see section VIII. Recommendations and important notes.

11. Incubate the DNA Purification Micro Spin Column at room temperature for 2 min.

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

13. Remove the DNA Purification Micro Spin Column. 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. 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 150 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 minicolumn placed in a collection tube. Centrifuge for 1 min at 11-15k x g.
   ▲ If the volume of the mixture exceeds 800 μl in total, discard the flow-through after centrifugation, then reuse the collection tube and transfer the remaining mixture into the same minicolumn.

5. Transfer the DNA Purification Micro Spin Column to a new Collection Tube.

6. Add 500 μl Wash Buffer and centrifuge for 30 s at 11-15k x g.

7. Discard the filtrate and reuse the Collection Tube.
8. Add **500 μl Wash Buffer** and centrifuge for 30 s at 11-15k x g.

9. Discard the filtrate and reuse the Collection Tube.

10. 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.

11. Discard the Collection Tube and flow-through and carefully transfer the DNA Purification Micro Spin Column to a sterile, 1.5 ml Eppendorf microcentrifuge tube.

12. Add **≥5 μl Elution Buffer**, pre-heated to 70°C, directly onto the purification minicolumn membrane.

    ▲ It is essential to apply the elution buffer precisely onto the centre of the membrane.

    ▲ Other buffer volumes may be used. For instructions, see section VIII. Recommendations and important notes.

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

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

15. Remove the DNA Purification Micro Spin Column. 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.
### XIII. TROUBLESHOOTING

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low yield of purified DNA</td>
<td>Ineffective DNA binding to the membrane.</td>
<td>Ensure the mixture is yellow after adding the CB and GB Buffers. If the colour turns pink, add 10 µl of 3 M sodium acetate, pH 5.2.</td>
</tr>
<tr>
<td>Incomplete DNA elution from the membrane.</td>
<td>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.</td>
<td></td>
</tr>
<tr>
<td>The pH of the water used for elution is lower than 7.0.</td>
<td>Use Elution Buffer for DNA elution.</td>
<td></td>
</tr>
<tr>
<td>Ethanol was not added to the Wash Buffer.</td>
<td>Ensure that 96-100% ethanol was added to the Wash Buffer before use.</td>
<td></td>
</tr>
<tr>
<td>Incomplete agarose slice dissolution.</td>
<td>Extend the incubation at 50°C until the agarose slice is completely lysed. After lysis, incubate the sample for an additional 5 minutes.</td>
<td></td>
</tr>
<tr>
<td>Column becomes clogged during purification</td>
<td>Incomplete agarose slice dissolution.</td>
<td>Extend the incubation at 50°C until the agarose slice is completely lysed. After lysis, incubate the sample for an additional 5 minutes.</td>
</tr>
<tr>
<td>DNA flows out of the lanes in the agarose gel</td>
<td>The purified DNA contains residual alcohol.</td>
<td>Repeat the isolation, giving particular attention to ensuring that no residual Wash Buffer is left in the purification column after centrifugation in step 8 in the clean-up protocol and step 10 in the gel-out protocol.</td>
</tr>
<tr>
<td>Blurred bands in the gel electrophoresis image</td>
<td>The running buffer contains nuclease.</td>
<td>Always use freshly prepared buffer for both the electrophoresis run and gel preparation. Store the gel fragment at +4°C, under DNase-free conditions, for no more than a few days.</td>
</tr>
<tr>
<td></td>
<td>The elution solution contains DNases.</td>
<td>Use fresh elution solution. If water is used instead of the Elution Buffer, ensure that it is DNase-free.</td>
</tr>
<tr>
<td>Inhibition of downstream enzymatic reactions</td>
<td>The running buffer for electrophoresis was contaminated.</td>
<td>Always use freshly prepared buffer for both the electrophoresis run and gel preparation.</td>
</tr>
<tr>
<td></td>
<td>The purified DNA contains residual salts.</td>
<td>Perform all centrifugation steps at room temperature. Ensure that there is no sediment in the Wash Buffer before use.</td>
</tr>
<tr>
<td></td>
<td>The purified DNA contains residual alcohol.</td>
<td>Repeat the isolation, giving particular attention to ensuring that no residual Wash Buffer is left in the purification column after centrifugation in step 8 in the clean-up protocol and step 10 in the gel-out protocol.</td>
</tr>
<tr>
<td>Incorrect DNA sequencing results.</td>
<td>Running buffer for electrophoresis was contaminated.</td>
<td>Always use freshly prepared buffer for both the electrophoresis run and gel preparation.</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>-----------------------------------------------------</td>
<td>------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Extensive exposure to the UV light.</td>
<td>Minimize the DNA's exposure time to the UV light during the excision from the gel procedure.</td>
<td></td>
</tr>
<tr>
<td>Equipment has been contaminated.</td>
<td>Clean the scalpel or razor blade and transilluminator surface prior to gel slice excision.</td>
<td></td>
</tr>
</tbody>
</table>

**XIV. SAFETY INFORMATION**

**CB Buffer**

Hazard

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

**GB Buffer**

Hazard

H302, H312, H315, H319, H332, H335
P305+P351+P338, P280, P261, P405, P302+P352

**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. **H335** May cause respiratory irritation. **H336** May cause drowsiness or dizziness. **P210** Keep away from heat/sparks/open flames/hot surfaces. No smoking. **P233** Keep container tightly closed. **P261** Avoid breathing dust/fume/gas/mist/vapours/spray. **P280** Wear protective gloves/protective clothing/eye protection/face protection. **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. **P405** Store locked up.