1. Excise DNA fragment out of the gel with a clean scalpel.
   ➔ Minimize the size of the gel slice by removing extra agarose.
2. Weigh the gel slice and transfer it to a clean tube.
3. Add 3 volumes of Extraction Buffer to 1 volume of gel slice.
   ➔ e.g., 300 µL of Extraction Buffer to 100 mg of gel. The gel slice should be ≤ 400 mg per assay.
   ➔ The Yellow color indicates a pH < 7.8
4. Incubate (5-10 min) at 50 °C until the gel melts and vortex the tube every 2-3 min during the incubation.
   ➔ If the color of the mixture becomes purple add 10 µl of 3 M sodium acetate (pH 5.0) and mix. The color will return yellow.
   Optional: if DNA Fragment < 500 bp or > 4 Kb, add 1 volume of isopropanol to 1 volume of gel and mix well.
5. Place a Smart Pure column into a collection tube (2mL)
6. Apply Sample on the SmartPure column.
7. Centrifuge for 1 min at 6 000 × g.
8. Discard the flow-through.
   ➔ If the sample volume is higher than 750 µL, reload the remaining volume on the same SmartPure column and spin again.
9. Add 500 µL of Extraction Buffer on the SmartPure column
10. Centrifuge for 30-60 sec at 12 000 × g.
11. Discard the flow-through.
12. Add 750 µL of Wash Buffer to the SmartPure column
13. Centrifuge for 30-60 sec at 12 000 × g.
14. Discard the flow-through.
   ➔ If the DNA will be used for salt sensitive applications, let the spin column stand for 2-5 min after the addition of the Wash Buffer before centrifuging.
15. Centrifuge for 1 min at 12 000 × g to remove residual liquid.
16. Transfer the SmartPure column to a sterile 1.5ml microcentrifuge tube.
17. Add 30-100 µL of Elution Buffer, H₂O or TE Buffer to the SmartPure column and let it stand for 1 min at room temperature.
   ➔ The volume of elution buffer can be adjusted according to needs (min 20 µL).
18. Centrifuge for 1 min at 12 000 × g to elute the DNA in the microcentrifuge tube.
   ➔ The purified DNA fragment can be used directly or stored at -20°C for long term storage.
The SmartPure Gel Kit ensures an easy, fast and effective extraction and purification of DNA fragments from up to 3% standard agarose gel in TAE or TBE buffer.

**First use**

*Add to the SmartPure Wash Buffer 40 mL of ethanol and mix well.*

**Kit components** (100 preps)

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>SmartPure Extraction Buffer</td>
<td>170 mL</td>
</tr>
<tr>
<td>SmartPure Wash Buffer</td>
<td>2x10 mL</td>
</tr>
<tr>
<td>SmartPure Elution Buffer</td>
<td>20 mL</td>
</tr>
<tr>
<td>SmartPure Column</td>
<td>100</td>
</tr>
</tbody>
</table>

**Material not supplied**

→ Sterile 1.5 mL microcentrifuge tubes  
→ 10/100/1000 µL tips  
→ Microcentrifuge (14 000 x g)  
→ Vortex mixer  
→ Absolute ethanol  
→ Isopropanol

**Technical Information**

<table>
<thead>
<tr>
<th>Feature</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column Volume</td>
<td>750 µL</td>
</tr>
<tr>
<td>Sample Volume</td>
<td>up to 400 mg gel slice</td>
</tr>
<tr>
<td>DNA size range</td>
<td>from 60 bp to 23 kb</td>
</tr>
<tr>
<td>Elution Recovery</td>
<td>≥ 99%</td>
</tr>
<tr>
<td>Working time</td>
<td>16 min/2 samples</td>
</tr>
<tr>
<td>Agarose Gel</td>
<td>Standard or high/low melting agarose</td>
</tr>
<tr>
<td>Electrophoresis buffer</td>
<td>TAE or TBE buffer</td>
</tr>
<tr>
<td>Incubation temperature</td>
<td>50°C for low-melting agarose, 55°C for standard agarose.</td>
</tr>
</tbody>
</table>

**Shipping & Storage conditions**

The SmartPure Gel Kit is shipped at room temperature.

The SmartPure Gel Kit is stable for 18 months if stored in a dry environment at room temperature (15°C-25°C).

**Troubleshooting**

**No recovery**

Please check whether the ethanol had been added to the Wash Buffer bottle.

**Low recovery**

1] The extraction buffer is acidic. The pH may increase during the gel melting process and could lead to inefficient DNA binding. Please add 0.1 volume 3M sodium acetate (pH5.0).

2] A non fresh electrophoresis buffer may lead to a low recovery.

3] Incubate the Elution Buffer at 30–60°C to increase the yields.

**Absorbance problem**

Please use the Elution Buffer to adjust zero value and dilute the sample.