

PCR CLEAN-UP**1 Sample preparation**

For volumes <30 μ l, adjust volume to 50–100 μ l with water.

Mix 1 volume of sample with 2 volumes of Binding Buffer CB.

2 Bind DNA

Place an ISOLATE II PCR and Gel Column in a 2ml Collection Tube and load sample.

Centrifuge 30s at 11,000 x g and discard flow-through.

Reuse collection tube for step 3.

3 Wash silica membrane

Add 700 μ l Wash Buffer CW to ISOLATE II PCR and Gel Column.

Centrifuge 30s at 11,000 x g.

Discard flow-through and place column back into Collection Tube.

Recommended: Repeat washing step to minimize chaotropic salt carry-over.

4 Dry silica membrane

Centrifuge 1 min at 11,000 x g, to remove residual ethanol.

Place ISOLATE II PCR and Gel Column in a 1.5ml microcentrifuge tube (not supplied).

5 Elute DNA

Add 15–30 μ l Elution Buffer C directly onto silica membrane.

Incubate at room temperature for 1 min.

Centrifuge 1 min at 11,000 x g.

DNA EXTRACTION FROM AGAROSE GELS

1 **Excise and dissolve gel slice**

Using a clean scalpel excise DNA fragment from gel.

Remove excess agarose, determine weight of gel slice and transfer into a clean tube.

Add 200µl Binding Buffer CB per 100mg of 2% agarose gel*.

** For gels containing >2% agarose, double the volume of Binding Buffer CB.*

Incubate sample at 50°C for 5–10 min, vortexing sample briefly every 2–3 min until gel slice is completely dissolved.

2 **Bind DNA**

Place ISOLATE II PCR and Gel Column in a 2ml Collection Tube and load sample.

Centrifuge 30s at 11,000 x g and discard flow-through.

Reuse collection tube for step 3.

3 **Wash silica membrane**

Add 700µl Wash Buffer CW to ISOLATE II PCR and Gel Column.

Centrifuge 30s at 11,000 x g.

Discard flow-through and place column back into collection tube.

Recommended: Repeat washing step to minimize chaotropic salt carry-over.

4 **Dry silica membrane**

Centrifuge 1 min at 11,000 x g, to remove residual ethanol.

Place ISOLATE II PCR and Gel Column in a 1.5ml microcentrifuge tube (not supplied).

5 **Elute DNA**

Add 15–30µl Elution Buffer C directly onto silica membrane.

Incubate at room temperature for 1 min.

Centrifuge 1 min at 11,000 x g.