

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 2 mL 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 1.5 mL 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 200 μ L Binding Buffer CB per 100 mg 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 2 mL 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 1.5 mL 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.