CYDR01-Gel Extraction Kit

Cat#: CYDR01

❖ Kit Contents and Storage

Kit Contents	Storage	50 Preps (CYDR0101)	100 Preps (CYDR0102)
Buffer DD	RT	50 ml	100 ml
Buffer WB	RT	15 ml Add indicated ethanol before first use	25 ml
Elution Buffer	RT	15 ml	20 ml
DNA Binding Columns	RT	50	100

All reagents, when stored at the indicated temperatures, are stable for 12 months.

❖ Description

This protocol is designed to extract and purify DNA of 100 bp to 10 kb from standard or low-melt agarose gels in TAE or TBE buffer. Up to 400 mg agarose can be processed per spin column. Simply add the specially formulated Buffer DD to dissolve the gel slice containing your DNA sample. After loading to the column, DNA binds selectively to silica membrane in the presence of the chaotropic salt guanidine thiocyanate. Bound DNA is purified in a series of rapid wash-and-spin steps to remove contaminants, and then eluted using a low salt solution. This simple method eliminates the need for organic solvent extractions and DNA precipitation, allowing for rapid purification of many samples simultaneously.

❖ Features

1. For purification of up to 10 µg PCR products, 100 bp to 10 kb.
2. Quick and easy recovery of ultra-pure DNA from agarose gels.
3. Colored Gel Dissolving buffer help visual identification of dissolving, binding and washing.

❖ Procedure

❖ Note:

⇨ **Before the first use, add the indicated amount of ethanol into Buffer WB bottles, mix well, and mark the bottle with a check.**

1. Excise the DNA fragment from the agarose gel with a clean, sharp scalpel.

   Note: Minimize gel volume by visualizing DNA and cutting the smallest possible gel

slice.

2. Place excised agarose gel slice in a sterile 1.5 ml microcentrifuge tube. Determine gel mass by first pre-weighing the tube, and then re-weighing the tube with the excised gel slice.
3. Add 3 volumes of Buffer DD to 1 volume of gel (100 mg~100 µl).
   For example, add 300 µl of Buffer DD to each 100 mg of gel. For >2% agarose gels, add 6 volumes of Buffer DD. The maximum amount of gel slice per DNA bind column is 400 mg; for gel slices >400 mg use more than one column.
4. Incubate at 56°C for 10 min (or until the gel slice has completely dissolved). To help dissolve gel, mix by vortexing the tube every 2–3 min during the incubation.
   IMPORTANT: Solubilize agarose completely. For >2% gels, increase incubation time.
5. After the agarose gel slice is completely dissolved, add 150µl isopropanol for every 100 mg agarose gel slice to the tube. Vortex thoroughly.
   For example, if the agarose gel slice is 100 mg, add 150 µl isopropanol. This step increases the yield of DNA fragments <500 bp and >4 kb. For DNA fragments between 500 bp and 4 kb, addition of isopropanol has no effect on yield. Do not centrifuge the sample at this stage.
6. Pipet the mixture into the spin column placed in a 2 ml collection tube (provided). Centrifuge at 13,000 rpm for 60 s. Discard flow-through and place the column back in the same tube.
   The maximum volume of the column reservoir is 720 µl. For sample volumes of more than 720 µl, simply load and spin again.
7. Add 600 µl Buffer WB, and centrifuge at 12,000 rpm for 30 s. Discard the flow-through.
8. Repeat Step 7 with another 600 µl Buffer WB.
9. Place the spin column back into the same collection tube. Centrifuge the empty column at 13,000 rpm for 2 min to completely remove ethanol from the column.
   Note: It is important to dry the membrane of the spin column, since residual ethanol may interfere with subsequent reactions. This centrifugation step ensures that no residual ethanol will be carried over during the following elution.
10. Place the column in a clean 1.5 ml microcentrifuge tube. Add 50 µl of Elution Buffer (Optional: pre-warm the water to 70–90 °C will increase the DNA yield) to the center of the column membrane. Incubate at room temperature for 2-3 min, and centrifuge at 12,000 rpm for 1 min to elute the DNA.

Note: Using a smaller volume (minimum 30µl) of Elution Buffer will **result in a** higher concentration.

Optional: Put eluate back to the spin column to repeat elution once. This increases concentration of DNA about 10-15%.

❖ **Procedure for PCR purification**

1. Add 5 volumes of Buffer DD to 1 volume of the PCR sample and mix. It is not necessary to remove mineral oil or kerosene.
   For example, add 500 µl of Buffer DD to 100 µl PCR sample (not including oil).
2. Pipet the mixture into the spin column placed in a 2 ml collection tube (provided). Centrifuge at 13,000 rpm for 30-60 s. Discard flow-through and place the column back in the same tube.
3. Add 600 µl Buffer WB, and centrifuge at 12,000 rpm for 30 s. Discard the flow-through.
4. Repeat Step 3 with another 600 µl Buffer WB.
5. Place the spin column back into the same collection tube. Centrifuge the empty column at 13,000 rpm for 2 min to completely remove ethanol from the column.
   Note: It is important to dry the membrane of the spin column, since residual ethanol may interfere with subsequent reactions. This centrifugation step ensures that no residual ethanol will be carried over during the following elution.
6. Place the column in a clean 1.5 ml microcentrifuge tube. Add 50 µl of Elution Buffer (Optional: pre-warm the water to 70–90°C will increase the DNA yield) to the center of the column membrane. Incubate at room temperature for 2-3 min, and centrifuge at 12,000 rpm for 1 min to elute the DNA.
   Note: Use smaller volume (minimum 30µl) of Elution Buffer will obtain higher concentration.
   Optional: Put eluate back to the spin column to repeat elution once. This increases concentration of DNA about 10-15%.

This product is furnished for LABORATORY RESEARCH USE ONLY.
Not for diagnostic or therapeutic use.