GeneKlean Gel Recovery & PCR CleanUp Kit

Cat. Number: 21004

Storage: Store at Room Temperature.

Kit Contents

GeneKlean Gel Recovery & PCR CleanUp Kit	300 Reactions
GeneKlean Column with 2 mL Collection Tube	300
Binding Solution B	300mL
Wash Solution	72mL
Elution Buffer	30mL
Sodium Acetate Solution	3 x 1mL
This Datasheet	1 copy

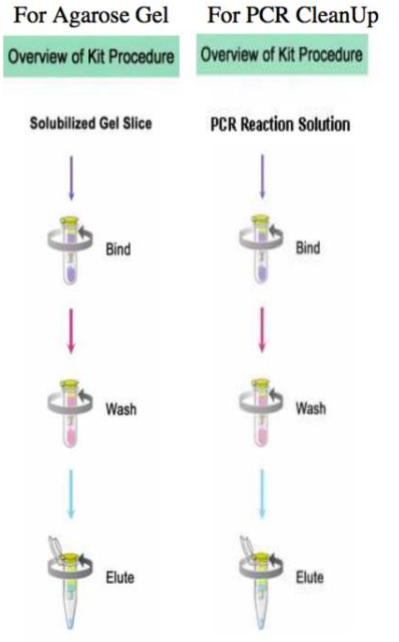
Reagent Preparation

- Before use, add 96mL of 100% ethanol to 24mL of Wash Solution. If Wash Solution leaked during transportation, it is necessary to re-measure its volume, and determine the volume of ethanol should be added (ethanol:Wash Solution = 4:1).
- Elution Buffer is TE buffer at pH 8.0.

Description

GeneKlean Gel Recovery & PCR CleanUp Kit utilizes silica-gel based membrane, which selectively adsorbs up to 10ug of DNA fragments in the presence of specialized binding buffers. Nucleotides, enzymes, mineral oil and other impurities do not bind to the membrane. DNA fragments can be eluted readily with Elution Buffer. The kit can be used in recovering of DNA fragments from reaction solutions and agarose gel with high yield (80%). It is suitable to recover 60bp-50kb DNA fragments. No phenol/chloroform extraction or ethanol precipitation required.

Procedure



1. Add Binding Solution B
   - **For Agarose Gel Recovery:**
     Excise the DNA fragment from the agarose gel with a clean, sharp scalpel. Weigh the gel slice and transfer to a 1.5mL centrifuge tube. Add 500uL of Binding Solution B to 100mg of gel (100mg = 100uL).
   - **For PCR CleanUp:**
     Add 300uL of Binding Solution B to 100uL PCR sample (not including oil) and mix. It is not necessary to remove mineral oil.
     If PCR sample is less than 100uL, please supply sterile water to total volume 100uL.
2. Check the color of the mixture
   - **For Agarose Gel Recovery:**
     Incubate at 50~60°C for 10 min. To help dissolve gel, mix by vortex every 2~3 min during the incubation. For high concentration agarose gel (1.5~2.0%), 1mL of Binding Solution B per 100mg of agarose gel should be added. The mixture should be yellow when agarose is completely dissolved.
     IMPORTANT: Be sure to dissolve gel completely. For >2% gel, increase incubation time.
   - **For PCR CleanUp:**
     Check that the color of the mixture is yellow (similar to Binding Buffer B without the PCR sample).
     If the color of the mixture is orange or violet, add 10uL of 3 M sodium acetate at pH 5.2 and mix. The color of the mixture will turn to yellow.

3. Place a spin column in a 2mL collection tube. Transfer the above mixture solution to the spin column. Let it stand for 2 min. Spin at 12000g for 1 min and discard the flow-through in the tube.
4. Add 500uL of Wash Solution, and spin at 12000g for 1 min. Discard the solution in the tube.
5. Repeat step 4. Spin at 12000g for additional 1 min to remove residual Wash Solution.
6. Place the column in a new clean 1.5mL centrifuge tube. Add 20-50uL of ddH2O (or Elution Buffer) to the center part of the membrane of the column and incubate at 37°C or 50°C for 2min. Spin at 12000g for 1 min to elute DNA. Store the DNA elution in –20°C freezer. If the plasmid is used for PCR, ddH2O is recommended.

Troubleshooting

Symptoms	Probable Cause	Suggestions
Low DNA recovery	Not enough binding solution was used .	**For Agarose Gel Recovery:** Add 3 volumes of Binding Solution B to gel slice. For gels containing more than 2% agarose, add 6 volumes of Binding Solution B. After gel is completely dissolved, add 1 volume of isopropanol and mix. **For PCR CleanUp:** Add Binding Solution B of 3 times of PCR Solution volume. If the DNA fragment is less than 200bp or more than 6kb, please add 0.6 times of isopropanol and mix.
	Gel slice not completely dissolved	Check that incubation temperature is at 50ºC, mix by vortex or inverting the tubes every 2 min. Make sure the gel slice completely dissolved.
	The Wash Solution did not contain ethanol.	Before use, add 96 ml 100% ethanol to 24 ml Wash Solution and mix well. Put a check mark in the box on the cap of the Wash Solution bottle.
	The dissolved gel solution was highly basic.	If the color of the dissolved gel solution is violet, add 5 to 10 µl of 3M sodium acetate (pH 5.0) and mix. The color of the mixture should be yellow for efficient binding.
	Other elution solution is used.	Elution Buffer is 2.5 mM Tris-HCl pH 8.5. TE buffer (pH 8.0) or water can also be used, but yield may be slightly lower.
	Gel slice too large	Repeated loadings of the dissolved gel solution to the same column will reduce the total yield of DNA. Trim the gel slice to remove extra agarose to reduce the size and weight of gel slice.