Catalog no. PC-GEK10a, PC-GEk10b
Size: 50 Reactions & 250 Reactions

Storage: Room Temperature*
Shipping: Room Temperature.
Shelf Life: 12 Months

Components	50 rxns PC-GEK10a	250 rxns PC-GEK10b
Gel Extraction Buffer (GEB)	75 ml	400 ml
Wash Buffer (WB) concentrated	10 mL	50 mL
Elution Buffer (EB)	5 mL	25 mL
Columns	50 nos.	250 nos.

Description

Procyto Labs Pvt. Ltd. Gel Extraction Kit offers a quick, convenient, and economical way to isolate DNA fragments from standard or low-melt Agarose gels following gel analysis. As a result of the buffer system included in the kit, DNA fragments that have been solubilized on gel slices easily bind to the spin column. The DNA samples are washed using wash buffers in order to remove agarose gel, salts, and other impurities. The purified DNA is eluted in small volumes for further processing. Each purification column has a total binding capacity of up to 20 µg of DNA and the entire procedure takes less than 15 min.

Applications

- High-quality sequencing
- Restriction digestion
- Labeling
- Ligation
- Cloning

Principle:

A DNA fragment is excised from an agarose gel, placed in a microcentrifuge tube, dissolved in binding buffer, and kept in dry bath. One volume of Isopropanol will be added upon complete dissolution of agarose gel. In the Gel Extraction Buffer, chaotropic agents are added, which dissolve agarose, denature proteins, and help DNA bind to the silica membrane. A simple wash step removes impurities. Using the elution buffer, purified DNA is eluted from the column. Following the recovery of DNA, downstream applications can be performed.

Important Notes

- Before the initial use of the kit, dilute the Wash Buffer (WB) (concentrated) with ethanol (96-100%):

Components	50 rxns PC-GEK10a	250 rxns PC-GEK11b
Wash Buffer (WB) concentrated	10 mL	50 mL
Ethanol	40 mL	200 mL
Total	50 mL	250 mL

After adding the ethanol, mark the check box on the bottle to indicate the completed step.

- Examine the Gel Extraction Buffer for precipitates before each use. Re-dissolve any precipitate by warming the solution to 37 °C and cooling it to 25 °C.
- Wear gloves when handling the Gel Extraction Buffer as this solution contains irritants

Certificate of Analysis

The kit was tested in the extraction of DNA fragments from an agarose gel according to the protocol described in the manual. The quality of the purified DNA was evaluated spectrophotometrically, by agarose gel electrophoresis, digestion with restriction enzymes.

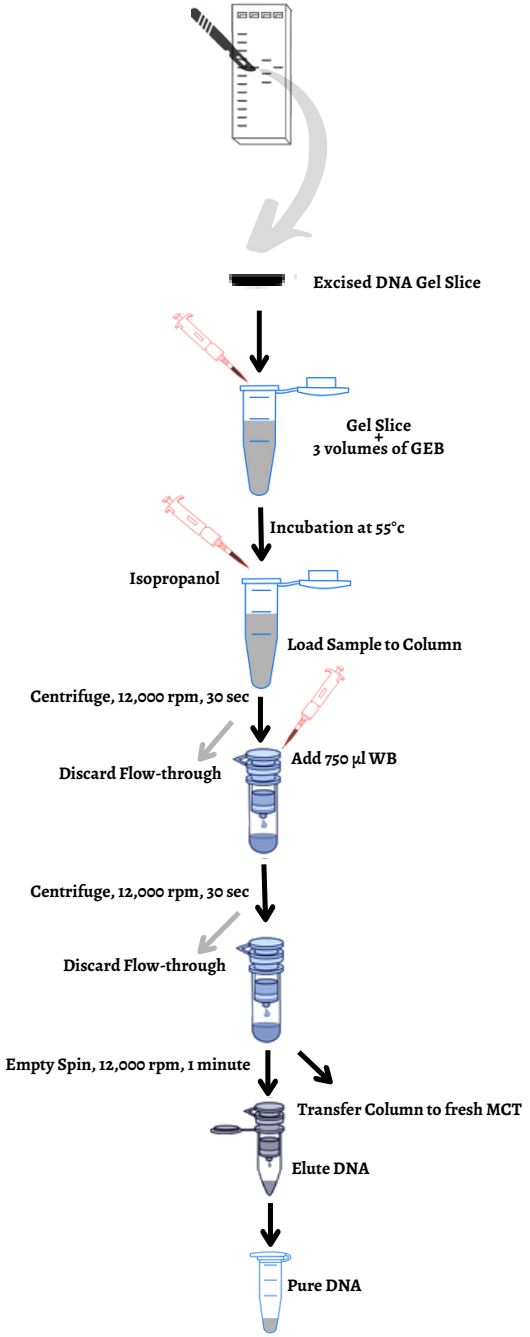
Quality authorized by:  Anjani Kumar Upadhyay

Protocol

Notes before starting

Pre-heat the Elution Buffer (EB) at 70°C

1. Cut the DNA fragment from the agarose gel with a clean scalpel.
2. Weigh the gel slice in a tube. Add 3 volumes of Buffer GEB to 1 volume of gel (100 mg gel ~ 100 µl).
3. Incubate at 55°C until the gel slice has completely dissolved. Vortex every 2-3 minutes to help dissolve the gel.
4. Add 1 gel volume of isopropanol **(not provided with the kit)** to the sample and mix.
5. Load sample to the spin column provided. Centrifuge at 12,000 rpm for 30 seconds. Discard the flow-through.
6. Add 750µl of Wash Buffer (WB) to the Column, and centrifuge at 12,000 rpm for 30 seconds. Discard the flow-through.
7. To remove the residual buffer, centrifuge the empty Column at 12,000 rpm for 1 minute.
8. Place the Column in a new 1.5ml microcentrifuge tube (not provided).
9. Add 30µl – 50 µl of pre-heated Elution Buffer (EB) to the center of the Column, let stand for 2 minutes, and centrifuge at 12,000rpm for 1 minute. **Ensure that the elution buffer is dispensed directly onto the membrane for complete elution of DNA.**
10. Analyze the purified DNA on an agarose gel.



Troubleshooting

Problem	Possible Cause and Solution
Yield of DNA is low	**Inefficient DNA binding** Verify that a 1:3 volume of Gel Extraction Buffer is added to the excised agarose gel. Ensure the solutions are mixed well. **Inefficient membrane wash** Ensure that the recommended volume of ethanol has been added to the Wash Buffer (concentrated) before first use. **Inefficient DNA elution** Elution buffer should be added straight to the membrane's center, not to the purification column's sides. Make sure to cover the membrane's surface entirely with the 30–50 µl of elution buffer you use. When purifying greater quantities of DNA, increase the Elution Buffer volume twice or carry out two elution cycles. (e.g., >15 µg). Ensure the membrane has received no more wash buffer after step 6 of the protocol. The wash buffer can be eliminated with an additional minute of centrifugation time. **PCR reaction mixture does not contain DNA** Check for the presence and yield of the PCR product by running an aliquot of the reaction on an agarose gel.
Downstream reactions are unsuccessful	**Presence of residual ethanol** In step 6 of the Protocol, ensure all residual wash buffer is removed from the membrane. Wash buffer can be removed more effectively with a longer centrifugation time. **Inefficient membrane wash** Check to see that the purification column has not been overfilled during the wash step and that no wash buffers have been left behind. Centrifugation flow-through should always be discarded.
DNA does not remain in an agarose gel well	In step 6 of the Protocol, ensure all residual wash buffer is removed from the membrane. Longer centrifugation time can aid in removal of wash buffer.