Catalog no. PC-DCK09a, PC-DCK09b
Size: 50 Reactions & 250 Reactions

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

Kit Content:

Components	50 rxns PC-DCK09a	250 rxns PC-DCK09b
DNA Binding Buffer (DBB)	28 mL	150 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.'s DNA clean-up kit is a fast, convenient and economical way to purify DNA fragments (50 bp - 10 Kb). In less than ten minutes, our product allows you to complete a rapid protocol. A PCR reaction mixture, enzyme mixture, or other reaction mixture that contains primers, dNTPs, and unincorporated labeled nucleotides is removed by this method. Furthermore, it uses a silica-based membrane technology, which facilitates easy resin manipulations and eliminates the need for toxic phenol-chloroform extractions.

Applications

- High-quality sequencing
- Microassay analysis
- Cloning
- Gene Silencing
- Restriction Digestion
- Enzymatic modifications
- Library Construction

Principle:

In a purification column, the DNA is mixed with a binding buffer. A chaotropic agent in the binding buffer denatures proteins and promotes DNA binding to the silica membrane in the column. Impurities can be removed with a simple wash step. Following the purification of the DNA, the elution buffer is used to elute the purified DNA. Using the recovered 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-DCK09a	250 rxns PC-DCK09b
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.

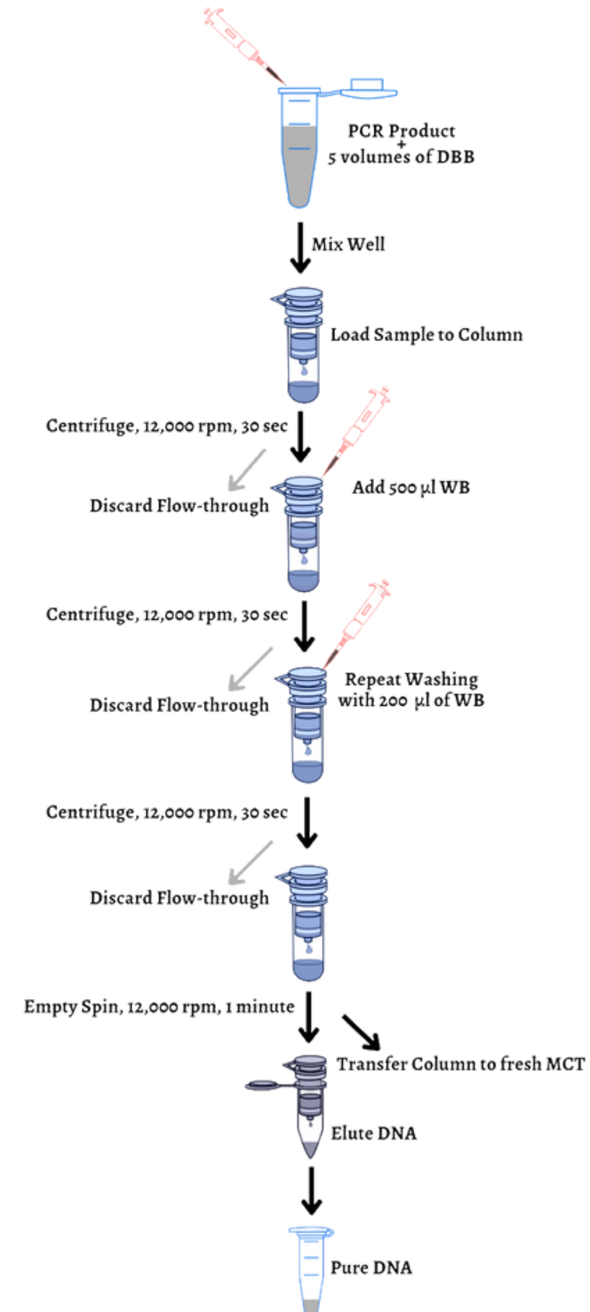
- Examine the Binding Buffer for precipitates before each use. Re-dissolve any precipitate by warming the solution to 37 °C and cooling it to 25 °C.
- Wear gloves when handling the Binding Buffer as this solution contains irritants.

Certificate of Analysis

The kit was tested in the purification of PCR products according to the protocol described in the manual. The quality of the purified DNA was evaluated spectrophotometrically, by agarose gel electrophoresis, and digestion.

Quality authorized by: Anjani Kumar Upadhyay

Protocol



Notes before starting

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

1. Add 5 volumes of DNA Binding Buffer (DBB) to one volume of PCR sample in a fresh microcentrifuge tube. Mix thoroughly (vortex briefly). **For example, for 50µl of PCR sample, add 250µl of DBB.**
2. Transfer the entire content to the Column (provided with the kit).
3. Centrifuge at 12,000 rpm for 30 seconds. Discard the flow-through.
4. Add 500µl of Wash Buffer (WB) to the Column and centrifuge at 12,000 rpm for 30 seconds. Discard the flow-through.
5. Add 200 µl of Wash Buffer (WB) to the Column. Centrifuge at 12,000 rpm for 30 Seconds. Discard the flow-through.
6. To remove the residual buffer, centrifuge the empty Column at 12,000 rpm for 1 minute.
7. Place the Column in a new 1.5 ml microcentrifuge tube (not provided).
8. Add 30 µl – 50 µl of pre-heated Elution Buffer (EB) to the center of the ProFast Column, let stand for 2 minutes, and centrifuge at 12,000 rpm for 1 minute. **Ensure that the elution buffer is dispensed directly onto the membrane for the complete elution of DNA.**
9. 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 1:5 volume of Binding Buffer is added to the reaction mixture. 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.