PCR and DNA Fragment Purification Kit

Catalog No: D1091 (50 preps) D1092 (100 preps) D1093 (200 preps)

Kit Content

Content	D1091	D1092	D1093
Binding Buffer BD	20 ml	40 ml	80 ml
Wash Buffer PE	15 ml	15 mlx2	20 mlx3
Elution Buffer (10 mM Tris-HCl, pH 8.5)	2.5 ml	5 ml	10 ml
Spin Columns	50 each	100 each	200 each

Description

PCR and DNA Fragment Purification Kit is designed for rapid and efficient purification of DNA from PCR and other enzymatic reaction mixtures. The kit utilizes a proprietary silica-based membrane technology in the form of a convenient spin column, eliminating the need for tedious resin manipulations or toxic phenol-chloroform extractions. The PCR and DNA Fragment Purification Kit effectively removes primers, dNTPs, unincorporated labeled nucleotides, enzymes and salts from PCR and other reaction mixtures. The kit can be used for purification of DNA fragments from 50 bp to 40 kb with recovery rates up to 100%. Each purification column has a total binding capacity of up to 30µg of DNA and the entire procedure takes just 15 min. The purified DNA can be used in common downstream applications such as sequencing, restriction digestion, labeling, ligation, cloning, *in vitro* transcription, blotting or *in situ* hybridization.

Applications

Fast and efficient purification of DNA fragments ideal for use in all conventional molecular biology procedures including:

- conventional restriction digestion
- automated fluorescent or radioactive sequencing
- PCR
- in *vitro* transcription

Feature

- **Fast** - procedure takes only 15 min.
- **High Efficient** – up to 85% recoveries in the range of 50bp-40kb.
- **Convenient**-spin columns are capped and assembled with collection tubes.
- **High purity** - $OD_{260/280}$=1.7-1.9. Purified DNA is ready for downstream application such as PCR, restriction digestion.

Store

PCR and DNA Fragment Purification Kit can be stored for up to 12 months at room temperature (15-25°C) or at 4°C for storage periods longer than 12 months. Any precipitate in the buffers can be re-dissolved by incubating at 37°C before use.

Quality Control

The kit is tested in the purification of 50 bp and 1 kb PCR products according to the protocol. The quality of the purified DNA is evaluated spectrophotometrically, by agarose gel electrophoresis, digestion with restriction enzymes and automated fluorescent sequencing.

Note

• Prior to the initial use of the kit, dilute the Wash Buffer PE with ethanol (96-100%):

	D1091(50preps)	D1092(100preps)	D1093(200preps)
Wash Buffer PE	15 ml	15 ml ×2	20ml ×3
Ethanol	60 ml	60 ml ×2	80 ml ×2
Total Volume	75 ml	75 ml ×2	100 ml ×2

After the ethanol has been added, mark the check box on the bottle to indicate the completed step.
• Examine the Binding Buffer BD for precipitates before each use. Re-dissolve any precipitate by warming the solution to 37°C and cooling to 25°C.
• Wear gloves when handling the Binding Buffer BD as this solution contains irritants.
• If extracted DNA will be used directly for sequencing, freshly prepared electrophoresis buffers should be used both for gel preparation and for gel running
• All centrifugations should be carried out in a table-top microcentrifuge at>12000 g (10,000-14,000 rpm, depending on the rotor type)

Protocol

1. Transfer the PCR reaction mix or other enzymatic reaction mix to a new 1.5ml microcentrifuge tube. And add a 1:1 volume of Bing Buffer BD to the mixture (e.g. for every 100 µl of reaction mixture, add 100 µl of Binding Buffer). Mix thoroughly.
2. Transfer up to 800 µl of the solution from step 1 to the spin column. Incubate for 2 min.
3. Centrifuge for 1 min at 12,000 rpm. Discard the flow-through. **Note**. If the total volume exceeds 800 µl, the solution can be added to the column in stages. After the addition of 800 µl of solution, centrifuge the column for 30-60 s and discard flow-though. Repeat until the entire solution has been added to the column membrane.
4. Add 500 µl of Wash Buffer PE to the column. Centrifuge for 1 min at 12,000 rpm. Discard the flow-through and place the purification column back into the collection tube. **Note** Wash Buffer PE must previously dilute with ethanol (96-100%).
5. Repeat step 4 again.
6. Centrifuge the empty column for an additional 3 min to completely remove any residual wash buffer. **Note**. This step is essential as the presence of residual ethanol in the DNA sample may inhibit subsequent reactions.
7. Empty the Collection Tube and recentrifuge the column assembly for 1 minute with the microcentrifuge lid open (or off) to allow evaporation of any residual ethanol.
8. Place the spin column in a clean 1.5 ml microcentrifuge tube (not provided), and pipet 30-100 µl Elution Buffer TE (prewarm to 60°C) directly to the center of the column without touching the membrane. Incubate at room temperature for 2 min.

   Note

   • For low DNA amounts the elution volumes can be reduced to increase DNA concentration. An elution volume between 20-50 µl does not significantly reduce the DNA yield. However, elution volumes less than 10 µl are not recommended.

• If DNA fragment is >10 kb, prewarm Elution Buffer to 60°C before applying to column.
• If the elution volume is 10 µl and DNA amount is >5 µg, incubate column for 1 min at room temperature before centrifugation.

9. Centrifuge for 1 minute at 12,000rpm. Discard the columns and store the microcentrifuge tube containing the eluted DNA at –20°C.

Note

• Elution buffer can be replaced by deionized water. But the PH should be 8.0-8.5.
• Prewarm Elution Buffer TE to 60°C can increase the yield of genomic DNA.