

Real Biotech Corporation

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HiYield™ Gel/PCR Fragments Extraction Kit Protocol Book

Maximum Yield / Mini Prep DNA Extration

Cat.No. YDF100 / YDF300

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HiYield™ Gel/PCR Fragments Extraction Kit

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HiYield™ Gel/PCR DNA Extraction Kit

**Cat.No. YDF100**

100 mini preps / kit
 DF Buffer: 80ml
 Wash Buffer (concentrated): 25 ml*
 Elution Buffer: 6 ml
 DF Columns: 100 pcs
 Collection Tubes: 100 pcs

Cat.No. YDF300

300 mini preps / kit
 DF Buffer: 240 ml
 Wash Buffer (concentrated): 50 ml**
 Elution Buffer: 30 ml
 DF Columns: 300 pcs
 Collection Tubes: 300 pcs

Sample: 200 mg Gel/100 µl PCR Solution
Yield: Gel 70-80%/PCR Recovery 80-90%
Effective Binding Capacity: Approx 10 µg
Effective Primer Removal: < 50 bp

Operation Time: 20 mins
Elution Volume: 15-50 µl
Seq. Cut-Off: 50bp-10kb

- * Add 100 ml ethanol (96~100%) to Wash Buffer before first use.
- ** Add 200 ml ethanol (96~100%) to Wash Buffer before first use.

Description

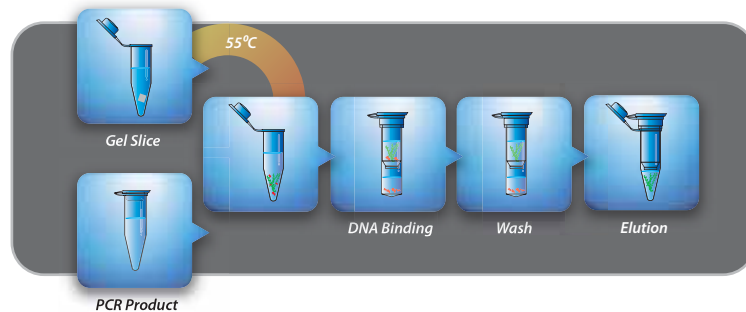
The HiYield™ Gel/PCR DNA Fragments Extraction Kit is designed to recover or concentrate DNA fragments (50 bp-10Kb) from agarose gels, PCR or other enzymatic reactions. The unique dual purpose application and high yield DNA/minicolumn make this kit exceptional value. The method uses a chaotropic salt, guanidine thiocyanate to dissolve agarose gel and denature enzymes. DNA fragments in chaotropic salt solution bind to the glass fiber matrix of the spin column. Following washing off contaminants, the purified DNA fragments are eluted by addition of low salt elution buffer or water. Salts, enzymes and unincorporated nucleotides are effectively removed from reaction mixtures without phenol extraction or alcohol precipitation.

Quality Control

The quality of HiYield™ Gel/PCR DNA Fragments Extraction Kit is tested on a lot-to-lot basis. The efficiency of DNA recovery is tested by isolation of DNA fragments of various sizes from either aqueous solution or agarose gel. The purified DNA is checked by agarose gel analysis.

Reference: Vogelstein, B., and Gillespie, D. (1979) Proc. Natl. Acad. Sci. USA 76, 615.

Note: DF Buffer contains guanidine thiocyanate which is harmful and an irritant agent. During operation, always wear a lab coat, disposable gloves, and protective goggles.

Gel Extraction Protocol**Gel Dissociation**

1. Excise the agarose gel slice containing relevant DNA Fragments and remove extra agarose to minimize the size of the gel slice.
2. Transfer up to 300 mg of the gel slice into a microcentrifuge tube (not provided).
3. Add 500 µl of DF Buffer to the sample and mix by vortexing.
4. Incubate at 55°C for 10-15 minutes until the gel slice has been completely dissolved. During incubation, invert the tube every 2-3 mins.

DNA Binding

5. Place a DF Column in a Collection Tube.
6. Apply 800 µl of the sample mixture from previous step into the DF Column.
7. Centrifuge at 6,000 x g (8,000 rpm) for 30 seconds.
8. Discard the flow-through and place the DF Column back in the Collection Tube.
9. If the sample mixture is more than 800 µl, repeat this DNA Binding Step.

Wash

10. Add 500 µl of Wash Buffer (ethanol added) into the DF column.
11. Centrifuge at 6,000 x g (8,000rpm) for 30 seconds.
12. Discard the flow-through and place the DF Column back in the Collection Tube.
13. Centrifuge again for 2 minutes at full speed (14,000rpm) to dry the column matrix.

DNA Elution

14. Transfer dried Column in a new microcentrifuge tube (not provided).
15. Add 15 µl - 30 µl of Elution Buffer or water into the center of the column matrix.
16. Stand for 2 minutes until Elution Buffer or water is absorbed by the matrix
17. Centrifuge for 2 minutes at full speed to elute purified DNA.

PCR Clean Up Protocol**Sample preparation**

1. Transfer up to 100 µl reaction product to a microcentrifuge tube (not provided).
2. Add 5 volumes of DF Buffer to 1 volume of the sample and mix by vortexing.

DNA Binding

3. Place a DF Column in a Collection Tube.
4. Apply the sample mixture from previous step into the DF Column.
5. Centrifuge at 6,000 x g (8,000 rpm) for 30 seconds.
6. Discard the flow-through and place the DF Column back in the Collection Tube.

Wash

7. Add 500 µl of Wash Buffer (ethanol added) into the DF column.
8. Centrifuge at 6,000 x g (8,000 rpm) for 30 seconds.
9. Discard the flow-through and place the DF Column back in the Collection Tube.
10. Centrifuge again for 2 minutes at full speed (14,000 rpm) to dry the column matrix.

DNA Elution

11. Transfer dried Column in a new microcentrifuge tube (not provided).
12. Add 15 µl of Elution Buffer or water into the center of the column matrix.
13. Stand for 2 minutes until Elution Buffer or water is absorbed by the matrix
14. Centrifuge for 2 minutes at full speed to elute purified DNA.

Troubleshooting

Problem	Possible Reason	Solution
Low recovery of DNA fragment	Size of DNA fragment is more than 5 kb	Use elution solution preheated to 60°C
Poor performance in downstream applications	Eluted DNA carries salt residues	Wash the column twice with Wash Buffer
	Eluted DNA carries salt residues	After washing, discard flow through and centrifuge column for 3-5 mins. Do not evaporate ethanol in oven as this may affect integrity of column.
Non-specific DNA fragment appears in eluted DNA	DNA fragment is denatured and becomes single-stranded	To re-anneal ss DNA, incubate tube at 95°C and cool slowly at room temp.
	Scalpel or razor blade used to excise gel is contaminated	Use a new or clean scalpel or razor blade to excise the gel
Gel slice difficult to dissolve	Used high percentage agarose gel (>2.5%) (not recommended)	Add double volume of buffer for gels >2.5%. Incubate with mixing every 1-2 minutes until complete dissolution.
	Gel slice is too big (> 300 mg)	Use more than one column for gel slice > 300mg
Low recovery of DNA fragment	Ineffective DNA elution	DNA elution may be ineffective in acidic conditions. Optimal elution pH is between 7.0 - 8.5
	Incomplete DNA elution	Make sure min. 30 ul is applied and elution buffer is applied to center of membrane. Allow time for full absorption to membrane prior to centrifugation
	TAE or TBE buffer is repeatedly used or of incorrect pH	Repeated use of TAE/TBE buffers will cause pH to increase. Use fresh TAE/TBE buffer.
	Overloaded column with agarose	Higher recovery is attained when smaller amounts of agarose are present. Minimize the size of the gel slice. If > 300mg, split sample and use another column.