USER GUIDE

HiYield™ Gel/PCR DNA Fragments Extraction Kit

Cat. AHGP100, AHGP300
Qty. 100r, 300r

ARROWTEC

Content and Storage

HiYield™ Gel/PCR DNA Fragments Extraction Kit
Cat.No. AHGP100/ AHGP300

Store at room temperature 15℃~25℃

Content.	**AHGP100**	**AHGP300**
Binding Buffer	80 ml	200 ml
Wash Buffer (concentrated)	25 ml *	40 ml **
Elution Buffer	10 ml	30 ml
2ml Collection Tube	100 pcs	300 pcs
Spin Cloumn	100 pcs	300 pcs

Sample: *200 mg Ge l / 100 µl PCR Solution*
Yields: *Gel 70 - 80 % / PCR Recovery 80 - 90 %*
Eective Binding Capacity: *Appro x 10 µg*
Eective Primer Removal : *< 25 bp*
Operation Time: *20 mins*
Elution Volume: *20-50 µl*
Seq. Cut-Off: *50bp - 10kb*
** Add 100ml ethanol (96-99.9%) to Wash Buffer prior to initial use.*
*** Add 160ml ethanol (96-99.9%) to Wash Buffer prior to initial use.*

Description

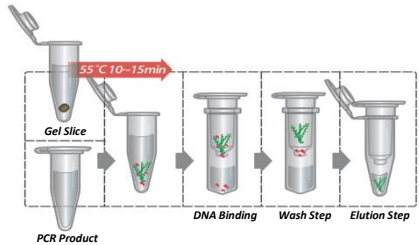
The HiYield™ Gel / PCR DNA Fragments Extraction Kit is designed to recover or concentrate DNA fragments (50bp - 10Kb) from agarose gels, PCR or other enzymatic reactions. The unique dual purpose application and high yield DNA / minicolumn make this kit exceptional value. The method uses a chaotropic 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.

Gel Extraction Protocol

OverView



Gel Dissociaton

1. Excise the agarose gel slice containing relevant DNA Fragments and remove extra agarose to minimize gel slice.
2. Transfer up to 300mg of the gel slice into a microcentrifuge tube (not provided).
3. Add 500 µl of Binding 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 Spin Column into a Collection Tube.
6. Apply 800µl of the sample mixture from previous step into the Spin Column.
7. Centrifuge at max. 10,000 xg (13,000 rpm) for 30 seconds.
8. Discard the flow-through and place the Spin Column back in the Collection Tube.
9. If the sample mixture is more than 800 µl, repeat this DNA Binding Step.

Wash

10. Add 600µl Wash Buffer (ethanol added) to the Spin Column.
11. Centrifuge at max. 10,000 xg (13,000 rpm) for 30 seconds.
12. Discard the flow-through and place the Spin Column back into the Collection Tube.

For TAE gels, proceed to step 13. For TBE gels, repeat Wash Steps 10-12.
Boric Acid is difficult to remove and can affect downstream applications, therefore double wash is recommended.

13. Centrifuge again for 2 minutes at max 10,000 xg (13,000 rpm) to dry the column matrix.

DNA Elution

14. Transfer dried Spin Column into a new microcentrifuge tube (not provided).
15. Add 20-50µl Elution Buffer or water to the center of the column matrix.
16. Allow to 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 volume of Binding Buffer to 1 volume of the sample and mix by vortexing.

▼ DNA Binding

3. Place a Spin Column into a Collection Tube.
4. Apply the sample mixture from previous step into the Spin Column.
5. Centrifuge at max. 10,000 xg (13,000 rpm) for 30 seconds.
6. Discard the flow-through and place the Spin Column back in the Collection Tube.

▼ Wash

7. Add 600µl of Wash Buffer (ethanol added) to the Spin Column.
8. Centrifuge at max. 10,000 xg (13,000 rpm) for 30 seconds.
9. Discard the flow-through and place the Spin Column back in the Collection Tube.
10. Centrifuge again for 2 minutes at max. 10,000 xg (13,000 rpm) to dry the column matrix.

▼ DNA Elition

11. Transfer dried Spin Column into a new microcentrifuge tube (not provided).
12. Add 20-50 µl Elution Buffer or water to the center of the column matrix.
13. Allow to stand for 2 minutes until Elution Buffer or water is absorbed by the matrix.
14. Centrifuge for 2 minutes at max. 10,000 xg (13,000 rpm) 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* ***Ineffective DNA elution*** *DNA elution may be in effective in acidic conditions. Optimal elution pH is between 7.0 - 8.5* ***Incomplete DNA elution*** *Make sure min . 30 µl 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 in correct 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.*
Poor performance in downstream applications	***Eluted DNA carries salt residues*** *Wash the column twice with Wash Buffer*
Non - Specific DNA fragment appears in eluted DNA	***DNA fragment is denatured and becomes single stranded*** *To re - anneal ssDNA , 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)*** *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*