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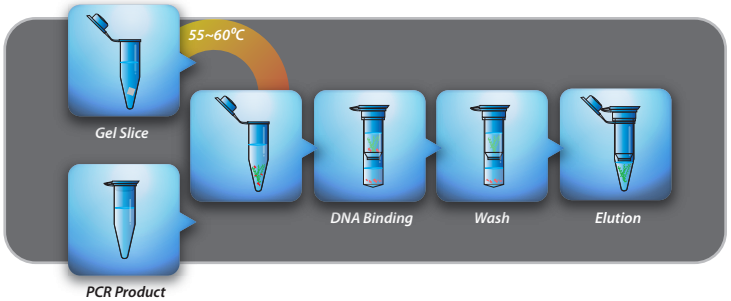
Real Genomics™

Store at room temperature 15°C~25°C



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HiYield™ Gel/PCR DNA Maxi Kit Protocol Book

Optimized for DNA Fragments between 200 bp and 10 kb

Cat. No. **YDM10 / YDM25**

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Real Genomics™

HiYield™ Gel/PCR DNA Maxi Kit

HiYield™ Gel/PCR DNA Maxi Kit

Store at room temperature 15°C~25°C

Cat. No. **YDM10**
10 maxi preps / kit
DF Buffer: 125 ml
W1 Buffer: 90 ml
Wash Buffer (concentrated): 25 ml *
Elution Buffer: 12 ml
DM Column: 10 pcs

Cat. No. **YDM25**
25 maxi preps / kit
DF Buffer: 290 ml
W1 Buffer: 210 ml
Wash Buffer (concentrated): 75 ml **
Elution Buffer: 30 ml
DM Column: 25 pcs

Sample: Up to 6 g of Agarose Gel, Up to 2 ml of PCR Product
Yield: Gel Recovery up to 90% / PCR Recovery up to 95%
Format: Spin Columns
Operation: Centrifuge or Vacuum
Operation Time: 40 Minutes for Gel Extraction / 25 Minutes for PCR Cleanup

* Add 100 ml of ethanol (96~100%) to 25 ml of Wash Buffer prior to the initial use.
** Add 100 ml of ethanol (96~100%) to 25 ml of Wash Buffer prior to the initial use.
Add 200 ml of ethanol (96~100%) to 50 ml of Wash Buffer prior to the initial use.

Description

HiYield™ Gel/PCR DNA Maxi Kit is designed to recover or concentrate DNA fragments (200 bp-10 kb) from agarose gels, PCR or other enzymatic reactions. The unique dual purpose application and high yield DM column make this kit exceptional value. Salts, enzymes and unincorporated nucleotides could be effectively removed from reaction mixtures without phenol extraction or alcohol precipitation. Typically, recoveries are up to 90% for gel extraction and up to 95% for PCR cleanup. For small base pair DNA fragments (50-200 bp), please choose HiYield™ Gel/PCR Small DNA Extraction Kit. For large base pair DNA fragments (>8 kb), please choose HiYield™ Gel/PCR Large DNA Extraction Kit.

Features

Up to 95% recovery of ready-to-use DNA.
Unique dual purpose application(Gel/PCR DNA Extraction).
Without phenol extraction or alcohol precipitation.

Applications

Purified DNA is ready for direct use in PCR, DNA Sequencing, DNA Library Screening and Analysis, Restriction Digestion, DNA Labeling, Preparation of PCR Probes for Microarrays, Ligation and Transformation.

Quality Control

The quality of HiYield™ Gel/PCR DNA Maxi Kits are tested on a lot-to-lot basis. The efficiency of DNA recovery are 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 thiocyanante which is harmful and an irritant agent. During operation, always wear a lab coat, disposable gloves, and protective goggles.

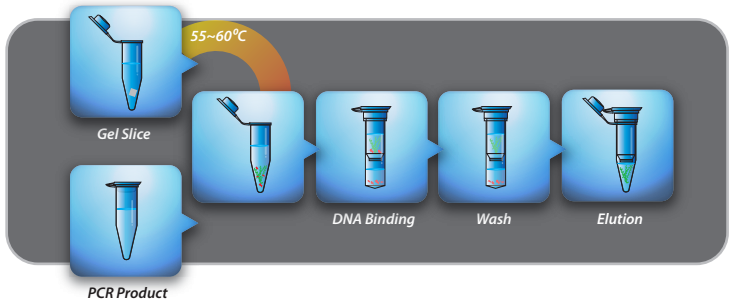
Gel Extraction Protocol

Additional requirements:

* 96% ~ 100% Ethanol.
* Sterile, DNase-free pipette tips and centrifuge tubes.

Things to do before starting:

Add absolute ethanol to the Wash Buffer prior to initial use (see the bottle label for details).



Gel Dissociaton

1. Excise the agarose gel slice containing relevant DNA fragments and remove any extra agarose to minimize the size of the gel slice. (It's better to make the gel with TAE buffer than TBE buffer, because TBE buffer might affect the downstream experiment.)
2. Transfer up to 6 g of the gel slice into a 15 ml centrifuge tube (not provided).
3. Add 10 ml of DF Buffer to the sample and mix by vortexing.
4. Incubate at 55~60°C for 10-15 minutes until the gel slice has been completely dissolved. During incubation, invert the tube every 2-3 minutes.
5. Cool the dissolved sample mixture to room temperature.

DNA Binding

6. Place a DM Column in a 50 ml centrifuge tube.
7. Apply 12 ml of the sample mixture from step 5 into the DM Column.
8. Centrifuge at full speed (approx. 13,000 rpm) for 2 minutes.
9. Discard the flow-through and place the DF Column back in the 50 ml centrifuge tube.
   (If the sample mixture is more than 12 ml, repeat this DNA Binding Step.)

Wash

10. Add 8 ml of W1 Buffer into the DM Column.
11. Centrifuge at full speed (approx. 13,000 rpm) for 2 minutes.
12. Discard the flow-through and place the DM Column back in the 50 ml centrifuge tube.
13. Add 12 ml of Wash Buffer (ethanol added) into the DM Column and let stand for 3 minutes.
14. Centrifuge at full speed (approx. 13,000 rpm) for 2 minutes.

15. Discard the flow-through and place the DM Column back in the 50 ml centrifuge tube.
    Note: For TAE gels, proceed to step 16. For TBE gel, repeat wash step 13-15. (Boric Acid is difficult to remove and can affect downstream applications, therefore double wash is recommended.)
16. Centrifuge again for 5 minutes at full speed (approx. 13,000 rpm) to dry the column matrix.

DNA Elution

17. Transfer the dried DM Column to a new 50 ml centrifuge tube (not provided).
18. Add 0.5 - 1 ml of Elution Buffer or TE into the center of the column matrix.
19. Let stand for 3 minutes until Elution Buffer or TE is absorbed by the matrix.
20. Centrifuge at full speed for 5 minutes to elute the purified DNA.

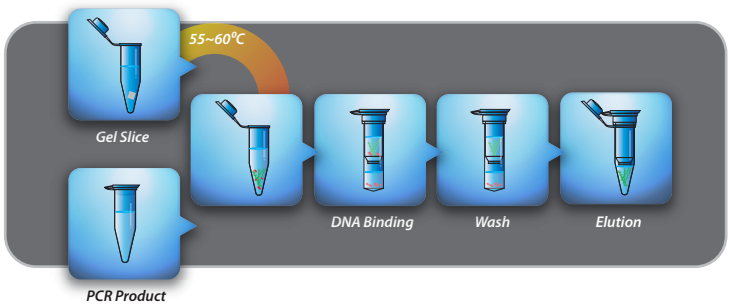
PCR Clean Up Protocol

Additional requirements:

* 96% ~ 100% Ethanol.
* Sterile, DNase-free pipette tips and centrifuge tubes.

Things to do before starting:

Add absolute ethanol to the Wash Buffer prior to initial use (see the bottle label for details).



HiYield™ Gel/PCR DNA Maxi Kit

Sample Preparation

1. Transfer up to 2 ml of a reaction product to a 15 ml centrifuge 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 DM Column in a 50 ml centrifuge tube.
4. Apply the sample mixture from step 2 into the DM Column.
5. Centrifuge at full speed (approx. 13,000 rpm) for 2 minutes.
6. Discard the flow-through and place the DM Column back in the 50 ml centrifuge tube.

Wash

7. Add 12 ml of Wash Buffer (ethanol added) into the center of the DM Column and let stand for 3 minutes.
8. Centrifuge at full speed (approx. 13,000 rpm) for 2 minutes.
9. Discard the flow-through and place the DM Column back in the 50 ml centrifuge tube.
10. Centrifuge again at full speed (approx. 13,000rpm) for 5 minutes to dry the column matrix.

DNA Elution

11. Transfer the dried DM Column to a new 50 ml centrifuge tube (not provided).
12. Add 0.5-1 ml of Elution Buffer or TE into the center of the column matrix.
13. Let stand for 3 minutes until Elution Buffer or TE is absorbed by the matrix.
14. Centrifuge for 5 minutes at full speed to elute the purified DNA.

Notes

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HiYield™ Gel/PCR DNA Maxi Kit

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