

GenepHlow™ PCR Cleanup Kit

DFC004 (4 Preparation Sample Kit)

DFC100 (100 Preparation Kit)

DFC300 (300 Preparation Kit)

Advantages

Convenient: includes pH indicator for easy determination of optimal pH and sodium acetate to adjust pH if it becomes too high following PCR product reaction

Sample: up to 100 µl of PCR products

Fragment Size: 100 bp-20 kb

Recovery: up to 95%

Format: PCR cleanup spin column

Operation Time: 10 minutes

Elution Volume: 20-50 µl

Kit Storage: dry at room temperature (15-25°C)

Table of Contents

| | |
|--|---|
| Introduction..... | 2 |
| Quality Control..... | 2 |
| Kit Components..... | 2 |
| Safety Measures..... | 3 |
| Quick Protocol Diagram and pH Indicator Information..... | 3 |
| PCR Cleanup Protocol Procedure..... | 4 |
| Troubleshooting..... | 5 |
| Test Data..... | 5 |
| Related Products..... | 6 |

Introduction

GenepHlow™ PCR Cleanup Kits were designed to recover or concentrate DNA fragments from PCR or other enzymatic reactions. This PCR cleanup kit includes a pH indicator as an optional addition to the PB binding buffer to ensure optimal pH and facilitate DNA binding. If pH exceeds the optimal level (>7.5), the color of the solution will appear purple instead of yellow. 3M Sodium Acetate (pH5.0) which is included with the kit, can then be added to the solution to adjust pH and return the color to yellow. Chaotropic salt is used to denature enzymes while DNA fragments are bound by the glass fiber matrix of the spin column. Contaminants are removed with a Wash Buffer (containing ethanol) and the purified DNA fragments are eluted by a low salt Elution Buffer, TE or water. The pH indicator, salts, enzymes and unincorporated nucleotides can be effectively removed from the reaction mixture without phenol extraction or alcohol precipitation and the purified DNA is ready for use in subsequent reactions.

Quality Control

The quality of the GenepHlow™ PCR Cleanup Kit is tested on a lot-to-lot basis by purifying DNA fragments of various sizes from PCR products. The purified DNA is analyzed by electrophoresis.

Kit Components

| Component | DFC004 | DFC100 | DFC300 |
|---|----------------|-------------------|-------------------|
| PB Buffer | 3 ml | 80 ml | 240 ml |
| pH Indicator | 15 µl | 360 µl | 1 ml |
| 3M Sodium Acetate (pH5.0) ¹ | N/A | 200 µl | 200 µl |
| Wash Buffer ² (Add Ethanol) | 1 ml (4 ml) | 25 ml (100 ml) | 50 ml (200 ml) |
| Elution Buffer | 1 ml | 6 ml | 30 ml |
| DFH Columns | 4 | 100 | 300 |
| 2 ml Collection Tubes | 4 | 100 | 300 |

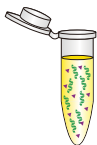
¹If the color of the mixture becomes purple instead of yellow then the pH is too high. 3M Sodium Acetate (pH5.0) can then be added to adjust pH and the color will return to yellow.

²Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

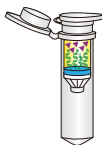


PB Buffer contains guanidine thiocyanate. During the procedure, always wear a lab coat, disposable gloves, and protective goggles.

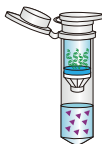
Quick Protocol Diagram



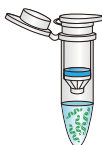
PB Buffer (pH \leq 7.5, yellow color when mixed with optional pH indicator) reaction of PCR or other enzymatic reaction product



DNA binding to membrane while contaminants remain suspended



Wash (removal of contaminants while DNA remains bound to membrane)



Elution of pure DNA which is ready for subsequent reactions

pH Indicator

Optimal pH



pH Too High



A pH indicator is included as an optional addition to the PB binding buffer to ensure optimal pH and facilitate DNA binding. If pH exceeds the optimal level (>7.5), the color of the solution will appear purple instead of yellow. 3M Sodium Acetate (pH5.0), which is included with the kit, can then be added to the solution to adjust pH and return the color to yellow.

GenepHlow™ PCR Cleanup Kit Protocol

Please read the entire instruction manual prior to starting the Protocol Procedure.

IMPORTANT BEFORE USE!

1. Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

2. It is not necessary to remove mineral oil or kerosene from the PCR sample prior to cleanup.

Additional Requirements

1.5 ml microcentrifuge tubes, absolute ethanol

PCR Cleanup Protocol Procedure

1. Sample Preparation

Optional: Add **1 ml of PB Buffer** and **4 µl of pH Indicator** to a new 1.5 ml microcentrifuge tube, then mix by shaking gently. The color will turn to yellow.

Transfer **100 µl of reaction product** to a 1.5 ml microcentrifuge tube. Add **5 volumes of PB Buffer (or PB Buffer premixed with pH Indicator)** to the sample then vortex.

If the mixture has turned from yellow to purple, add 10 µl of 3M sodium acetate (pH5.0) and mix thoroughly. This will adjust pH and the color will return to yellow.

2. DNA Binding

Place a **DFH Column** in a 2 ml Collection Tube. Transfer the sample mixture to the **DFH Column**. Centrifuge at 14-16,000 x g for 30 seconds. Discard the flow-through then place the **DFH Column** back in the 2 ml Collection Tube.

3. Wash

Add **600 µl of Wash Buffer (make sure absolute ethanol was added)** into the **DFH Column** and let stand for 1 minute. Centrifuge at 14-16,000 x g for 30 seconds then discard the flow-through. Place the **DFH Column** back in the 2 ml Collection Tube. Centrifuge at 14-16,000 x g for 3 minutes to dry the column matrix.

4. Elution

Transfer the dried **DFH Column** to a new 1.5 ml microcentrifuge tube. Add **20-50 µl of Elution Buffer¹**, TE² or water³ into the **CENTER** of the column matrix. Let stand for at least 2 minutes to allow Elution Buffer, TE or water to be completely absorbed. Centrifuge at 14-16,000 x g for 2 minutes at room temperature to elute the purified DNA.

¹Ensure that Elution Buffer (10 mM Tris-HCl, pH8.5 at 25°C) is added into the center of the DFH Column matrix and is completely absorbed.

²Using TE (10 mM Tris-HCl, 1 mM EDTA, pH8.0) for elution is beneficial as EDTA preserves DNA for long term storage. However, EDTA will affect PCR and other sensitive downstream applications. Ensure that TE is added into the center of the DFH Column matrix and is completely absorbed.

³If using water for elution, ensure the water pH is ≥ 8.0 . ddH₂O should be fresh as ambient CO₂ can quickly cause acidification. Ensure that water is added into the center of the DFH Column matrix and is completely absorbed. DNA Eluted in water should be stored at -20°C to avoid degradation.

Troubleshooting



Low Yield

Incomplete Wash Buffer preparation.

Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

Incorrect DNA Elution step.

Ensure that Elution Buffer, TE or water is added into the **CENTER** of the DFH Column matrix and is completely absorbed. If DNA fragments are larger than 5 kb, use pre-heated Elution Buffer, TE, or water (60~70°C). If using water for elution, ensure the water pH is ≥ 8.0 . ddH₂O should be fresh as ambient CO₂ can quickly cause acidification.

GenePflow™ PCR Cleanup Functional Test Data

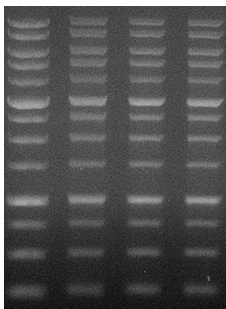


Figure 1. PCR product DNA fragments ranging from 250 bp-10 kb were extracted using the GenePflow™ PCR Cleanup Kit (lane 1, 2, 3). The purified DNA concentration from a 50 µl eluate was confirmed by spectrophotometer and analyzed by electrophoresis on a 1% agarose gel.

M = Geneaid 1 Kb DNA Ladder (control, total DNA = 1073 ng)

| Test | DNA Conc. | 260/280 | Yield | Recovery |
|------|------------|---------|---------|----------|
| 1 | 19.7 ng/µl | 1.86 | 985 ng | 91.8% |
| 2 | 20.3 ng/µl | 1.85 | 1015 ng | 94.6% |
| 3 | 20.5 ng/µl | 1.86 | 1025 ng | 95.5% |

Related DNA/RNA Extraction Products

| Plasmid DNA Purification | | |
|--|--------------------|------------------------|
| Product | Package Size | Catalogue Number |
| Presto™ Mini Plasmid Kit | 100/300 preps | PDH100/300 |
| Presto™ Midi Plasmid Kit | 25 preps | PIF025 |
| Presto™ Midi Plasmid Kit (Endotoxin Free) | 25 preps | PIFE25 |
| High-Speed Plasmid Mini Kit (10-50 Kb) | 100/300 preps | PDL100/300 |
| High-Speed Plasmid Advance Kit (50-100 ml) | 25 preps | PA025 |
| Geneaid™ Midi Plasmid Kit | 25 preps | PI025 |
| Geneaid™ Midi Plasmid Kit (Endotoxin Free) | 25 preps | PIE25 |
| Presto™ Plasmid DNA Concentration Kit | 250/500/1000 preps | PC0250/500/1000 |
| Geneaid™ Maxi Plasmid Kit | 10/25 preps | PM010/25 |
| Geneaid™ Maxi Plasmid Kit (Endotoxin Free) | 10/25 preps | PME10/25 |
| Presto™ 96 Well Plasmid Kit | 4/10 x 96 preps | 96PDV04/10, 96PDC04/10 |
| Post Reaction DNA Purification | | |
| Product | Package Size | Catalogue Number |
| GenepHlow™ Gel Extraction Kit | 100/300 preps | DFG100/300 |
| GenepHlow™ PCR Cleanup Kit | 100/300 preps | DFC100/300 |
| GenepHlow™ Gel/PCR Kit | 100/300 preps | DFH100/300 |
| Gel/PCR DNA Fragments Extraction Maxi Kit | 10/25 preps | DM010/025 |
| Small DNA Fragments Extraction Kit | 100/300 preps | DF101/301 |
| Presto™ Max Gel/PCR Kit (Large DNA Fragments) | 100/300 preps | DFL100/300 |
| Presto™ 96 Well PCR Cleanup Kit | 4/10 x 96 preps | 96DFH04/10 |
| G-25 Gel Filtration Desalting Column | 50 rxns | CG025 |
| G-50 Gel Filtration Dye Terminator Removal Column | 50 rxns | CG050 |
| 96-Well G-50 Gel Filtration Plate | 4/10 x 96 rxns | CGP04/10 |
| Genomic DNA Extraction and Purification | | |
| Product | Package Size | Catalogue Number |
| Genomic DNA Mini Kit (Blood/Cultured Cell) | 100/300 preps | GB100/300 |
| Genomic DNA Midi Kit (Blood/Cultured Cell) | 25 preps | GDI25 |
| Genomic DNA Maxi Kit (Blood/Cultured Cell) | 10/25 preps | GDM10/25 |
| Genomic DNA Mini Kit (Tissue) | 50/100/300 preps | GT050/100/300 |
| gSYNC™ DNA Extraction Kit | 50/100/300 preps | GS050/100/300 |
| Genomic DNA Mini Kit (Plant) | 100 preps | GP100 |
| Geneaid™ DNA Isolation Kit (Blood) | 100/1,000 rxns | GEB100/01K(+) |
| Geneaid™ DNA Isolation Kit (Bacteria) | 300/3,000 rxns | GEE300/03K(+) |
| Geneaid™ DNA Isolation Kit (Tissue) | 150/1,500 rxns | GET150/1.5K(+) |
| Geneaid™ DNA Isolation Kit (Cultured Cell) | 150/1,500 rxns | GEC150/1.5K(+) |
| GENEzo1™ DNA Reagent Plant | 100/200 rxns | GR100/200 |
| Presto™ Mini gDNA Yeast Kit | 100/300 preps | GBY100/300 |
| Presto™ Mini gDNA Bacteria Kit | 100/300 preps | GBB100/101/300/301 |
| Genieus™ Micro DNA Extraction Kit | 100/300 preps | GMB100/300 |
| Presto™ Buccal Swab gDNA Extraction Kit | 100/300 preps | GSK100/300 |
| Presto™ 96 Well Genomic DNA Extraction Kit | 4/10 x 96 preps | 96GB004/010 |
| Presto™ 96 Well Genomic DNA Extraction Kit (Plant) | 4/10 x 96 preps | 96GP004/010 |
| DNA RNA Purification | | |
| Product | Package Size | Catalogue Number |
| Presto™ DNA RNA Extraction Kit | 50/100 preps | DR050/100 |

Related DNA/RNA Extraction Products

| RNA Extraction and Purification | | |
|--|--|------------------|
| Product | Package Size | Catalogue Number |
| Total RNA Mini Kit (Blood/Cultured Cell) | 50/100/300 preps | RB050/100/300 |
| Total RNA Mini Kit (Tissue) | 50/100/300 preps | RT050/100/300 |
| Total RNA Mini Kit (Plant) | 50/100/300 preps | RP050/100/300 |
| Presto™ Mini RNA Bacteria Kit | 50/100/300 preps | RBB050/100/300 |
| Presto™ Mini RNA Yeast Kit | 50/100/300 preps | RB050/100/300 |
| Presto™ 96 Well Total RNA Extraction Kit | 4/10 x 96 preps | 96RB004/010 |
| miRNA Isolation Kit | 50/100 preps | RMI050/100 |
| GENEzol™ Reagent | 50/100/200 rxns | GZR050/100/200 |
| GENEzol™ TriRNA Bacteria Kit | 50/100 rxns | GZB050/100 |
| GENEzol™ TriRNA Pure Kit | 50/100/200 preps | GZX050/100/200 |
| TriRNA Pure Kit | 50/100/200 preps | TRP050/100/200 |
| RNA Pure Kit | 50/100 preps | PR050/100 |
| GENEzol™ 96 Well TriRNA Pure Kit | 4/10 x 96 preps | 96TR004/010 |
| Virus DNA/RNA Purification | | |
| Product | Package Size | Catalogue Number |
| Plant Virus RNA Kit | 50/100 preps | PVR050/100 |
| Viral Nucleic Acid Extraction Kit II | 50/100/300 preps | VR050/100/300 |
| Viral Nucleic Acid Extraction Kit III | 50/100/300 preps | VI050/100/300 |
| Presto™ 96 Well Viral DNA RNA Extraction Kit | 4/10 x 96 preps | 96VR004/010 |
| Cloning | | |
| Product | Package Size | Catalogue Number |
| Elite™ TA Cloning Kit | 20 rxns | TA020 |
| Elite™ TA Cloning Vector | 20 rxns | TV020 |
| Elite™ T4 DNA Ligase | 300 U | TL100 |
| Elite™ Competent Cells (XL1-Blue) | >5 x 10 ⁷ , 100 µl x 10, 80 | CX571, CX578 |
| Elite™ Competent Cells (XL1-Blue) | >2 x 10 ⁸ , 100 µl x 10, 80 | CX281, CX288 |
| Elite™ Competent Cells (XL1-Blue) | >5 x 10 ⁸ , 100 µl x 10, 80 | CX581, CX588 |
| Elite™ Competent Cells (DH5α) | >1 x 10 ⁸ , 100 µl x 10, 80 | CD181, CD188 |
| Elite™ Competent Cells (DH5α) | >3 x 10 ⁸ , 100 µl x 10, 80 | CD381, CD388 |
| Elite™ Competent Cells (DH5α) | >1 x 10 ⁹ , 100 µl x 10, 80 | CD191, CD198 |
| Elite™ Competent Cells BL21(DE3) | >2 x 10 ⁷ , 100 µl x 10, 80 | CB271, CB278 |
| Elite™ Competent Cells (JM109) | >5 x 10 ⁷ , 100 µl x 10, 80 | CJ571, CJ578 |
| Elite™ Competent Cells (JM109) | >1 x 10 ⁸ , 100 µl x 10, 80 | CJ181, CJ188 |
| DNA Ladders and Markers | | |
| Product | Package Size | Catalogue Number |
| 100 bp DNA Ladder | 50 µg, 500 µl | DL004 |
| 1 Kb DNA Ladder | 50 µg, 500 µl | DL005 |
| 100 bp DNA Marker | 50 µg, 500 µl | DL006 |
| 1 Kb DNA Marker | 50 µg, 500 µl | DL007 |
| 100 bp + 50 bp DNA Marker | 50 µg, 500 µl | DL008 |
| Loading Dye (6X) | 10/100 ml | LD010/100 |
| LE Agarose | 500 g | AGA500 |

For additional product information please visit www.geneaid.com. Thank you!

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