Geneaid

Instruction Manual
Ver. 04.10.13
For Research Use Only

GenepHlow™ Gel/PCR Kit

DFH004 (4 Preparation Sample Kit)
DFH100 (100 Preparation Kit)
DFH300 (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 gel dissociation or PCR product reaction
Sample: up to 300 mg of agarose gel, up to 100 µl of PCR products
Fragment Size: 70 bp-20 kb
Recovery: up to 90% for gel extraction, up to 95% for PCR cleanup
Format: gel extraction/PCR cleanup spin column
Operation Time: 20 minutes for gel extraction, 10 minutes for PCR cleanup
Elution Volume: 20-50 µl
Kit Storage: dry at room temperature (15-25ºC) for up to 1 year

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Introduction

GenepHlow™ Gel/PCR Kits were designed to recover or concentrate DNA fragments from agarose gel, PCR or other enzymatic reactions. Gel/PCR Buffer (yellow color indicating optimal pH≤7.5) is premixed with a pH indicator to ensure optimal pH, facilitate DNA binding and allow for easy observation of undissolved agarose gel. 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 dissolve agarose gel and 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™ Gel/PCR Kit is tested on a lot-to-lot basis by purifying DNA fragments of various sizes from agarose gel. The purified DNA is analyzed by electrophoresis.

Kit Components

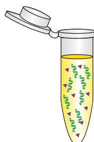
Component	DFH004	DFH100	DFH300
Gel/PCR Buffer	3 ml	80 ml	240 ml
3M Sodium Acetate (pH5.0)[1]	N/A	200 µl	200 µl
W1 Buffer	2 ml	45 ml	130 ml
Wash Buffer[2] (Add Ethanol)	1 ml (4 ml)	25 ml (100 ml)	50 ml + 25 ml (200 ml) (100 ml)
Elution Buffer	1 ml	6 ml	30 ml
DFH Columns	4	100	300
2 ml Collection Tubes	4	100	300

[1]If the color of the mixture becomes purple instead of yellow once the gel slice is dissolved completely or following PCR product reaction, 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.

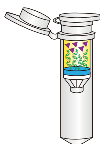
[2]Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

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

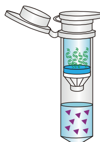
Quick Protocol Diagram



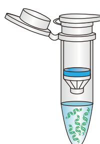
Gel/PCR Buffer (pH≤7.5, yellow color, premixed with pH indicator) reaction of gel slice or PCR product



DNA binding to blue membrane while contaminants remain suspended



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



Elution of pure DNA which is ready for subsequent reactions

pH Indicator

Optimal pH

pH Too High

A pH indicator is premixed with the Gel/PCR binding buffer to ensure optimal pH, facilitate DNA binding and allow for easy observation of undissolved agarose gel. 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™ Gel/PCR Kit Protocol 1 of 3

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. Record the weight of an empty 1.5 ml microcentrifuge tube for the gel dissociation step. Once the gel has been transferred to the tube, record the weight again. Subtract the empty tube weight from the total weight to determine the actual gel weight.
3. Perform gel purification when primer dimers are highly visible or add an additional 80% ethanol wash to avoid primer dimer contamination.

Additional Requirements

1.5 ml microcentrifuge tubes, absolute ethanol

Gel Extraction Protocol Procedure

1. Gel Dissociation

Cut the agarose gel slice containing relevant DNA fragments and remove any extra agarose to minimize the size of the gel slice. Transfer **up to 300 mg of the gel slice** to a 1.5 ml microcentrifuge tube. Add **500 µl of Gel/PCR Buffer** to the sample then mix by vortex. Incubate at 55-60ºC for 10-15 minutes or until the gel slice is completely dissolved. During incubation, invert the tube every 2-3 minutes. If the color of 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. Cool the dissolved sample mixture to room temperature.

NOTE: If using less than 300 mg of gel slice, Gel/PCR Buffer does not need to be scaled. If using more than 300 mg of gel slice, separate it into multiple 1.5 ml microcentrifuge tubes.

2. DNA Binding

Place a **DFH Column** in a 2 ml Collection Tube. Transfer **800 µl of 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.

NOTE: If the sample mixture is more than 800 µl, repeat the DNA binding step.

3. Wash

Add **400 µl of W1 Buffer** into the **DFH Column**. 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. 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 Buffer**[1], TE[2] or water[3] 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.

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

[2]Using 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.

[3]If using water for elution, ensure the water pH is ≥8.0. ddH_2O should be fresh as ambient CO_2 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 -20ºC to avoid degradation.

GenepHlow™ Gel/PCR Kit Protocol 2 of 3

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. Record the weight of an empty 1.5 ml microcentrifuge tube for the gel dissociation step. Once the gel has been transferred to the tube, record the weight again. Subtract the empty tube weight from the total weight to determine the actual gel weight.
3. Perform gel purification when primer dimers are highly visible or add an additional 80% ethanol wash to avoid primer dimer contamination.

Additional Requirements

1.5 ml microcentrifuge tubes, absolute ethanol

Gel Extraction Protocol Procedure for Sequencing

1. Gel Dissociation

Cut the agarose gel slice containing relevant DNA fragments and remove any extra agarose to minimize the size of the gel slice. Transfer **up to 300 mg of the gel slice** to a 1.5 ml microcentrifuge tube. Add **500 µl of Gel/PCR Buffer** to the sample then mix by vortex. Incubate at 55-60ºC for 10-15 minutes or until the gel slice is completely dissolved. During incubation, invert the tube every 2-3 minutes. If the color of 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. Cool the dissolved sample mixture to room temperature.

NOTE: If using less than 300 mg of gel slice, Gel/PCR Buffer does not need to be scaled. If using more than 300 mg of gel slice, separate it into multiple 1.5 ml microcentrifuge tubes.

2. DNA Binding

Place a **DFH Column** in a 2 ml Collection Tube. Transfer **800 µl of 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.

NOTE: If the sample mixture is more than 800 µl, repeat the DNA binding step.

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. 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 Buffer**[1], TE[2] or water[3] 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.

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

[2]Using 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.

[3]If using water for elution, ensure the water pH is ≥8.0. ddH_2O should be fresh as ambient CO_2 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 -20ºC to avoid degradation.

GenepHlow™ Gel/PCR Kit Protocol 3 of 3

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

Transfer **100 µl of reaction product** to a 1.5 ml microcentrifuge tube. Add **5 volumes of Gel/PCR Buffer** 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 and 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 Buffer**[1], TE[2] or water[3] 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.

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

[2]Using 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.

[3]If using water for elution, ensure the water pH is ≥8.0. ddH_2O should be fresh as ambient CO_2 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 -20ºC to avoid degradation.

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Troubleshooting

Low Yield

Agarose gel did not dissolve completely.

Ensure the agarose gel was melted/dissolved completely between 55-60ºC for 10-15 minutes, or until no gel is visible. If undissolved agarose remains in the sample, the DFH Column could clog and some DNA will be unrecoverable. DNA can be denatured if the incubation temperature exceeds 60ºC. If using more than 300 mg of agarose gel, separate it into multiple 1.5 ml microcentrifuge tubes.

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 (60~70ºC). If using water for elution, ensure the water pH is ≥8.0. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification.

Eluted DNA Does Not Perform Well In Downstream Applications

DNA was denatured (a smaller band appeared on gel analysis).

Ensure the agarose gel was melted/dissolved completely between 55-60ºC for 10-15 minutes, or until no gel is visible. DNA can be denatured if the incubation temperature exceeds 60ºC. Incubate the eluted DNA at 95ºC for 2 minutes then cool down slowly to re-anneal the denatured DNA.

Primer dimer contamination in the final PCR elution product.

Gel purification should be performed if primer dimers are visible in the agarose gel following PCR reactions. Simply cut the PCR product from the gel and avoid the primer dimer. Using an additional 80% ethanol wash will reduce primer dimer contamination when performing PCR cleanup.

GenepHlow™ Gel Extraction Functional Test Data

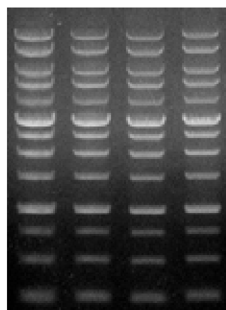

Figure 1. Gel slice DNA fragments ranging from 250 bp-10 kb were extracted using the GenepHlow™ Gel Extraction Kit (lane 1, 2, 3). The purified DNA from a 50 μl eluate was analyzed by electrophoresis on a 1% agarose gel.

M = Geneaid 1 Kb DNA Ladder (control, total DNA = 1100 ng)

Test	DNA Conc.	260/280	Yield	Recovery
1	18.2 ng/μl	1.84	910 ng	82%
2	18.7 ng/μl	1.82	935 ng	85%
3	19.2 ng/μl	1.82	960 ng	87.3%

GenepHlow™ PCR Cleanup Functional Test Data

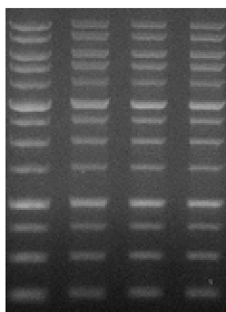

Figure 2. PCR product DNA fragments ranging from 250 bp-10 kb were extracted using the GenepHlow™ PCR Cleanup Kit (lane 1, 2, 3). The purified DNA concentration from a 50 μl eluate was confirmed by spectrophotometer and analyzed by electrophoresis on a 1% agarose gel.

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%

GenepHlow™ DNA Recovery Data

DNA Recovery (50 μl eluates)		
DNA Size	PCR Cleanup	Gel Extraction
90 bp	90%	77%
1.5 Kb	95%	90%
7.7 Kb	85%	79%
13 Kb	83%	77%

Table 1. The correlation between DNA fragment size and DNA recovery using the GenepHlow™ Kits to purify 1-3 μg of DNA.

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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 Plasmid Midi Kit	25 preps	PI025
Geneaid Plasmid Midi Kit (Endotoxin Free)	25 preps	PIE25
Geneaid Plasmid Maxi Kit	10/25 preps	PM010/25
Geneaid Plasmid Maxi Kit (Endotoxin Free)	10/25 preps	PME10/25
96-Well Plasmid Kit	4/10 x 96 preps	PDA04/10
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 Kit	100/300 preps	DF100/300
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
96-Well Gel/PCR DNA Extraction Kit	4/10 x 96 preps	DFP04/10
DNA Pure Kit	100/300 preps	DP100/300
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
Genomic DNA Maxi Kit (Plant)	10/25 preps	GPM10/25
Geneaid™ DNA Isolation Kit (Blood)	100/1,000 rxns	GEB100/01K
Geneaid™ DNA Isolation Kit (Bacteria)	100/1,000 rxns	GEE100/01K
Geneaid™ DNA Isolation Kit (Tissue)	100/1,000 rxns	GET100/01K
Geneaid™ DNA Isolation Kit (Cultured Cell)	100/1,000 rxns	GEC100/01K
GENEzol™ 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	GBB100/300
Geneius™ Micro DNA Extraction Kit	100/300 preps	GMB100/300
Presto™ Buccal Swab gDNA Extraction Kit	100/300 preps	GSK100/300

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	RBY050/100/300
96-Well Total RNA Extraction Kit	4/10 x 96 preps	RBP04/10
miRNA Isolation Kit	50 preps	RMI050
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
Virus DNA/RNA Purification		
Product	Package Size	Catalogue Number
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
96-Well Viral DNA/RNA Extraction Kit	4/10 x 96 preps	VNP04/10
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

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

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CERTIFICATE NO. QAIC/TW/50077

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