Geneaid

Instruction Manual
Ver. 02.10.17
For Research Use Only

Presto™ 96 Well Gel Extraction Kit

96DFG02 (2 x 96 well plates/kit)
96DFG04 (4 x 96 well plates/kit)
96DFG10 (10 x 96 well plates/kit)

Advantages

Sample: up to 300 mg of agarose gel per well
Fragment Size: 70 bp-20 kb
Recovery: up to 80% per well
Format: Presto™ Gel Extraction 96 Well Binding Plates for efficient vacuum filtration and centrifugation
Operation Time: 45 minutes
Elution Volume: 60 µl from 80 µl elution buffer volume, and 40 µl from 60 µl elution buffer volume
Kit Storage: dry at room temperature (15-25°C)

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Introduction

Presto™ 96 Well Gel Extraction Kits were designed to recover or concentrate DNA fragments from agarose gel. QG 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 Presto™ Gel Extraction 96 Well Binding Plate. 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 Presto™ 96 Well Gel Extraction 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

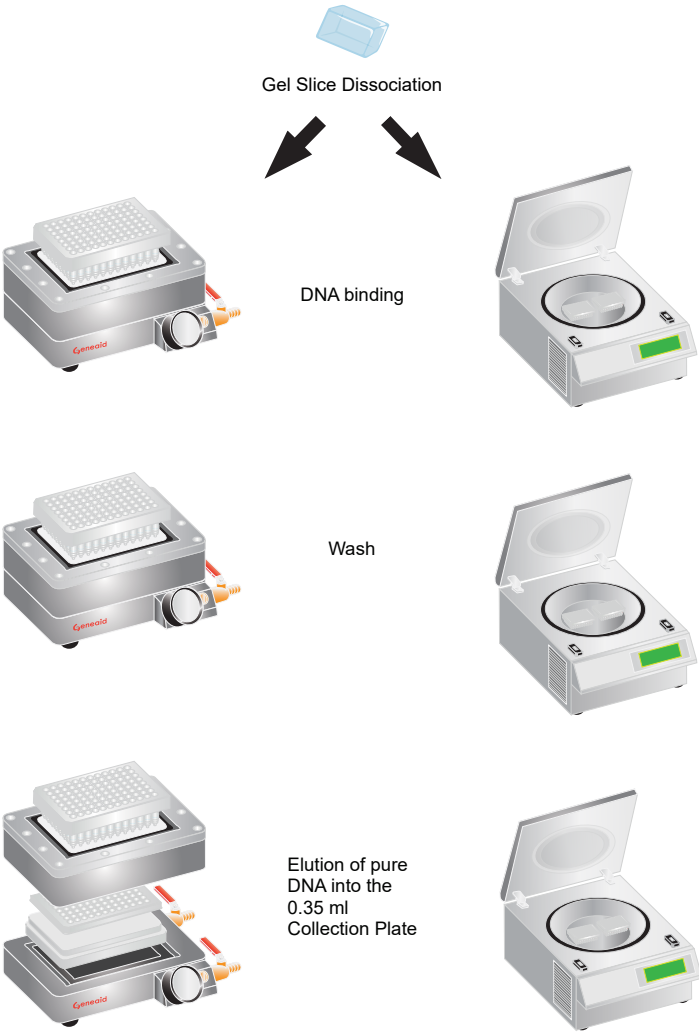
Component	96DFG02	96DFG04	96DFG10
QG Buffer	125 ml	240 ml	275 ml x 2
3M Sodium Acetate (pH5.0)[1]	2 ml	2 ml	2 ml
W1 Buffer	80 ml	160 ml	200 ml x 2
Wash Buffer[2] (Add Ethanol)	25 ml (100 ml)	50 ml (200 ml)	25 ml + 50 ml x 2 (100 ml) (200 ml x 2)
Elution Buffer	30 ml	60 ml	100 ml
Presto™ Gel Extraction 96 Well Binding Plates	2	4	10
0.35 ml Collection Plates	2	4	10
Adhesive Film	6	12	30

[1]If the color of the mixture becomes purple instead of yellow once the gel slice is dissolved completely 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.

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

Quick Vacuum and Centrifuge Protocol Diagram



pH Indicator

Optimal pH pH Too High

A pH indicator is premixed with QG 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.

Presto™ 96 Well Gel Extraction 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 important that the weight of the agarose gel slice does not exceed 300 mg. Please weigh each agarose gel slice before transferring to each well of the 96 Deep Well Plate.

3. Perform gel purification when primer dimers are highly visible or add an additional 80% ethanol wash to avoid primer dimer contamination.

Additional Requirements
absolute ethanol, 96 Deep Well Plate (optional), Square-well Block (optional)

Gel Extraction Vacuum Protocol Procedure

1. Vacuum Manifold Preparation

Place the waste tray on the manifold base then place the binding top plate on the manifold base. Place the **Presto™ Gel Extraction 96 Well Binding Plate** in the binding top plate aperture. Seal unused wells of the **Presto™ Gel Extraction 96 Well Binding Plate** with **Adhesive Film** then attach the vacuum manifold to a vacuum source.

2. 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 each well of a 96 Deep Well Plate. Add **500 µl of QG Buffer** to each gel sample. Dry the top of the 96 Deep Well Plate with paper towel. Seal the plate tightly with **Adhesive Film** then mix by vortex. Incubate the plate at 55-60ºC in an oven or water bath for 10-15 minutes or until all gel slices are completely dissolved. During incubation, invert the plate every 3 minutes.

NOTE: If the DNA fragments are larger than 5 kb, pre-heat the required Elution Buffer (80 µl per sample) to 60ºC (for Step 5 DNA Elution).

3. DNA Binding

Cool the sample mixture to room temperature then briefly centrifuge the plate at 2,000 x g to collect any sample mixture remaining on the **Adhesive Film**. Allow the centrifuge to reach 2,000 x g prior to stopping. Remove the **Adhesive Film** from the plate. If the color of the sample mixture has turned from yellow to purple, add **10 µl of 3M Sodium Acetate (pH5.0)** and mix thoroughly by pipetting. This will adjust pH of the sample mixture and the color will return to yellow. Transfer the sample mixture to each well of the **Presto™ Gel Extraction 96 Well Binding Plate**. Apply vacuum at 15 inches Hg until samples pass through completely (approximately 10 seconds) then turn off the vacuum.

4. Wash

Add **300 µl of W1 Buffer** to each well of the **Presto™ Gel Extraction 96 Well Binding Plate**. Apply vacuum at 15 inches Hg until **W1 Buffer** passes through completely (approximately 10 seconds) then turn off the vacuum. Add **600 µl of Wash Buffer (make sure ethanol was added)** to each well. Let stand for 1 minute then apply vacuum at 15 inches Hg until **Wash Buffer** passes through completely. Continue to apply vacuum for an additional 10 minutes to dry the membrane then turn off the vacuum.

5. Elution

Remove the **Presto™ Gel Extraction 96 Well Binding Plate** from the binding top plate aperture and blot the nozzles on a clean absorbent paper towel to remove residual ethanol. Remove the waste tray from the manifold base then place the collection plate spacer on the manifold base. Place a **0.35 ml collection plate** on top of the collection plate spacer. Place the binding top plate back on the manifold base then place the **Presto™ Gel Extraction 96 Well Binding Plate** back in the binding top plate aperture. Add **60-80 µl of Elution Buffer**[1], TE[2] or water[3] into the **CENTER** of each well of the **Presto™ Gel Extraction 96 Well Binding Plate**. Let stand for at least 3 minutes to ensure the Elution Buffer, TE or water is absorbed by the membrane. Apply vacuum at 15 inches Hg for 5 minutes then turn off the vacuum. Seal the **0.35 ml Collection Plate** with **Adhesive Film** and store the purified DNA at -20ºC. NOTE: The average eluate volume is 60 µl from 80 µl elution buffer volume, and 40 µl from 60 µl elution buffer volume.

[1]Ensure that Elution Buffer (10 mM Tris-HCl, pH8.5 at 25ºC) is added into the center of the well 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 well 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 well matrix and is completely absorbed. DNA eluted in water should be stored at -20ºC to avoid degradation.

Gel Extraction Centrifuge 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 each well of a 96 Deep Well Plate. Add **500 µl of QG Buffer** to each gel sample. Dry the top of the 96 Deep Well Plate with paper towel. Seal the plate tightly with **Adhesive Film** then mix by vortex. Incubate the plate at 55-60ºC in an oven or water bath for 10-15 minutes or until all gel slices are completely dissolved. During incubation, invert the plate every 3 minutes.

NOTE: If the DNA fragments are larger than 5 kb, pre-heat the required Elution Buffer (80 µl per sample) to 60ºC (for Step 4 DNA Elution).

2. DNA Binding

Cool the sample mixture to room temperature then briefly centrifuge the plate at 2,000 x g to collect any sample mixture remaining on the adhesive film. Allow the centrifuge to reach 2,000 x g prior to stopping. Remove the **Adhesive Film** from the plate. If the color of the sample mixture has turned from yellow to purple, add **10 µl of 3M Sodium Acetate (pH5.0)** then mix thoroughly by pipetting. This will adjust pH of the sample mixture and the color will return to yellow. Place the **Presto™ Gel Extraction 96 Well Binding Plate** on a new 96 Deep Well Plate. Transfer the sample mixture to each well of the **Presto™ Gel Extraction 96 Well Binding Plate**. Centrifuge the **Presto™ Gel Extraction 96 Well Binding Plate** and 96 Deep Well Plate together at 3,000 x g for 5 minutes. Discard the flow-through then place the **Presto™ Gel Extraction 96 Well Binding Plate** back on the 96 Deep Well Plate.

3. Wash

Add **300 µl of W1 Buffer** to each well of the **Presto™ Gel Extraction 96 Well Binding Plate**. Centrifuge the **Presto™ Gel Extraction 96 Well Binding Plate** and 96 Deep Well Plate together at 3,000 x g for 5 minutes. Discard the flow-through then place the **Presto™ Gel Extraction 96 Well Binding Plate** back on the 96 Deep Well Plate. Add **600 µl of Wash Buffer (make sure ethanol was added)** to each well of the **Presto™ Gel Extraction 96 Well Binding Plate**. Let stand for 1 minute then centrifuge the **Presto™ Gel Extraction 96 Well Binding Plate** and 96 Deep Well Plate together at 3,000 x g for 5 minutes. Discard the flow-through then place the **Presto™ Gel Extraction 96 Well Binding Plate** back on the 96 Deep Well Plate. Centrifuge the **Presto™ Gel Extraction 96 Well Binding Plate** and 96 Deep Well Plate together at 3,000 x g for an additional 10 minutes to dry the membrane.

4. Elution

Remove the **Presto™ Gel Extraction 96 Well Binding Plate** from the 96 Deep Well Plate and blot the nozzles on a clean absorbent paper towel to remove residual ethanol. Place the **Presto™ Gel Extraction 96 Well Binding Plate** on a **0.35 ml Collection Plate**. Add **60-80 µl of Elution Buffer**[1], TE[2] or water[3] to the **CENTER** of each well of the **Presto™ Gel Extraction 96 Well Binding Plate**. Let stand for at least 3 minutes to ensure the Elution Buffer, TE or water is absorbed by the membrane. Centrifuge the **Presto™ Gel Extraction 96 Well Binding Plate** and **0.35 ml Collection Plate** together at 3,000 x g for 5 minutes. Seal the **0.35 ml Collection Plate** with **Adhesive Film** and store the purified DNA at -20ºC. NOTE: The average eluate volume is 60 µl from 80 µl elution buffer volume, and 40 µl from 60 µl elution buffer volume.

[1]Ensure that Elution Buffer (10 mM Tris-HCl, pH8.5 at 25ºC) is added into the center of the well 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 well 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 well matrix and is completely absorbed. DNA eluted in water should be stored at -20ºC to avoid degradation.

Presto™ 96 Well Gel Extraction Kit Functional Test

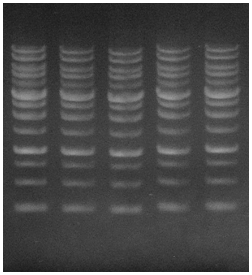
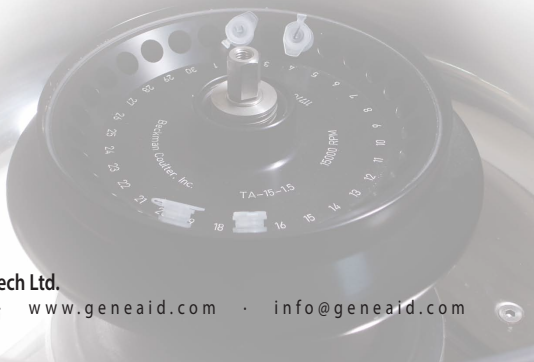


Figure 1. Gel slice DNA fragments ranging from 250 bp-10 kb were extracted using the Presto™ 96 Well Gel Extraction Kit (lane 1, 2, 3, 4). The purified DNA from a 60 µl eluate (chosen from 4 random wells) was analyzed by electrophoresis on a 0.8% agarose gel. M = Geneaid 1 Kb DNA Ladder (control, total DNA = 1100 ng)

	Sample	DNA	Total DNA	Recovery
1	Random Well	14 ng/µl	840 ng	76.4%
2	Random Well	13.9 ng/µl	834 ng	75.8%
3	Random Well	13.4 ng/µl	804 ng	73.1%
4	Random Well	14.6 ng/µl	876 ng	79.6%



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 96 well plate could clog and some DNA will be unrecoverable. DNA can be denatured if the incubation temperature exceeds 60ºC. Do not use more than 300 mg of agarose gel per well.

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 each well 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 reanneal 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.

Related DNA/RNA Purification 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
GenepHlow™ DNA Cleanup Midi Kit	100/300 preps	DFI100/300
GenepHlow™ DNA Cleanup Maxi Kit	10/25 preps	DFM010/025
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
Presto™ 96 Well Gel Extraction Kit	4/10 x 96 preps	96DFG04/10
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(+)
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	GBB100/101/300/301
Geneius™ Micro DNA Extraction Kit	100/300 preps	GMB100/300
Presto™ Buccal Swab gDNA Extraction Kit	100/300 preps	GSK100/300
Presto™ 96 Well Blood Genomic DNA Extraction Kit	4/10 x 96 preps	96GBP04/10
DNA RNA Purification		
Product	Package Size	Catalogue Number
Presto™ DNA/RNA/Protein Extraction Kit	50/100 preps	DRP050/100

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
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
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
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	DL007
1 Kb DNA Ladder	50 µg, 500 µl	DL006
Loading Dye (6X)	10/100 ml	LD010/100

PCR		
Product	Package Size	Catalogue Number
Ultra-Pure Taq DNA Polymerase	500 U	UT050
HiFi Taq DNA Polymerase	500 U	HT050
Ultra-Pure Taq PCR Master Mix	200/400 rxns	UTM200/400
Ultra-Pure Taq PCR Master Mix with Dye	100 rxns	TQMD100
dNTP Solution	10 mM each, 200 µl	DN200
dNTP Solution	25 mM each, 1 ml	DN1100
dNTP Set	100 mM 1 ml x 4	DN4400
dCTP	100 mM, 1 ml	DC1000
dATP	100 mM, 1 ml	DA1000
dGTP	100 mM, 1 ml	DG1000
dTTP	100 mM, 1 ml	DT1000
Enzymes		
Product	Package Size	Catalogue Number
Proteinase K	11/100 mg	PK000011/100
RNase A (50 mg/ml)	50/130/200/1500 µl	RA500050/130/200/1500
RNase A (10 mg/ml)	550/1000 µl	RA100550/1000
RNase A	100/250/550/1000 mg	RA0100/250/500/1000
Lysozyme	20/420/1220 mg	LY020/420/1220
Protein		
Product	Package Size	Catalogue Number
Prestained Protein Ladder V	500 µl	PL005
Protein Loading Dye (5X)	2 ml	PLD001
Dithiothreitol (DTT)	500 µl	DTT001
Reverse Protein Stain Kit	50/500 ml	PS050/500
Laboratory Equipment		
Product	Package Size	Catalogue Number
Micropestle	50 pcs/pkg	MP050
Microtube Rack	1 rack	A4MR080
PCR Sample Rack	1 rack	A4PR096
96-Well PCR Plate	5 plates/pkg	PN034
2 ml Collection Plate	1 plate	A4PD020
Presto™ Vac 96 Well Vacuum Manifold	1 set	VZF01/VZF03

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

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UKAS QUALITY MANAGEMENT

Registration Number 046

CERTIFICATE NO. QAIC/TW/50077

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