Geneaid

Instruction Manual
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For Research Use Only

Presto™ 96 Well Blood Genomic DNA Extraction Kit

96GBP02 (2 x 96 well plates/kit)
96GBP04 (4 x 96 well plates/kit)
96GBP10 (10 x 96 well plates/kit)

Advantages

Sample: up to 200 µl of whole blood, plasma, serum and up to 5 x 10^6 lymphocytes or cultured cells per well
Yield: up to 6 µg of genomic DNA from 200 µl of whole blood
Format: Presto™ gDNA 96 Well Binding Plate
Operation Time: 45 minutes
Elution Volume: 200~400 µl
Kit Storage: dry at room temperature (15-25°C)

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Introduction

The Presto™ 96 Well Blood Genomic DNA Extraction Kit was designed for high-throughput purification of genomic, mitochondrial and virus DNA from whole blood (fresh blood and frozen blood), plasma, serum, body fluids, lymphocytes and cultured cells. This DNA extraction kit uses proteinase K and chaotropic salt to lyse cells and degrade protein, allowing DNA to bind to the glass fiber matrix of the Presto™ gDNA 96 Well Binding Plate. Contaminants are removed using a Wash Buffer and the purified genomic DNA is eluted by a low salt Elution Buffer, TE or water. The procedure can be completed within 45 minutes without phenol/chloroform extraction or alcohol precipitation. The purified DNA (approximately 20-30 kb) is suitable for use in PCR or other enzymatic reactions.

Quality Control

The quality of the Presto™ 96 Well Blood Genomic DNA Extraction Kit is tested on a lot-to-lot basis by purifying genomic DNA from 200 µl of whole blood samples. The purified DNA is quantified with a spectrophotometer and analyzed by electrophoresis.

Kit Components

Component	96GBP02	96GBP04	96GBP10
GB Buffer	40 ml	100 ml	155 ml x 1 60 ml x 1
Proteinase K[1] (Add ddH_2O)	40 mg (4 ml)	80 mg (8 ml)	100 mg x 2 (10 ml)
W1 Buffer	80 ml	200 ml	200 ml x 2
Wash Buffer[2] (Add Ethanol)	25 ml (100 ml)	50 ml (200 ml)	25 ml x 1 (100 ml) 50 ml x 2 (200 ml x 2)
Elution Buffer	100 ml	100 ml x 2	100 ml x 4
Presto™ gDNA 96 Well Binding Plates	2	4	10
Microtubes (Racked)	2	2	2
Microtubes (8-strip)	12 x 2	12 x 6	12 x 18
Caps for Microtubes (8-strip)	72	72 x 2	72 x 5
96 Deep Well Plates[3]	2	2	2

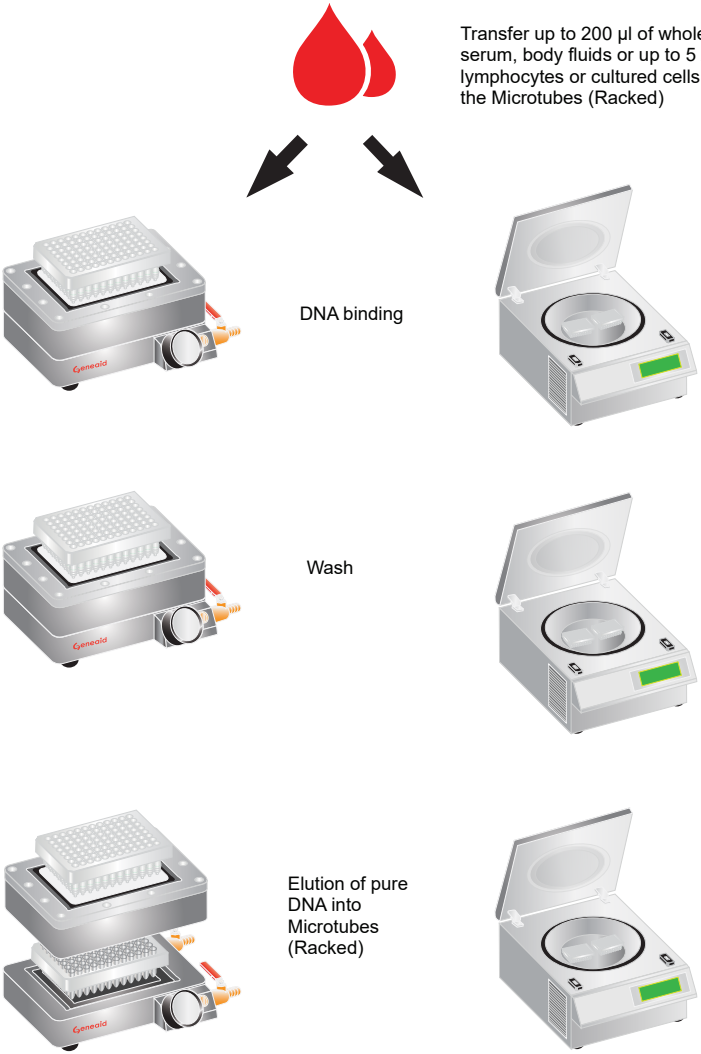
[1]Add ddH_2O pH7.0-8.5 (see the bottle label for volume) to Proteinase K then vortex to ensure Proteinase K is completely dissolved. Check the box on the bottle. For extended periods, the ddH_2O and Proteinase K mixture should be stored at 4ºC. Use only fresh ddH_2O as ambient CO_2 can quickly cause acidification.

[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.

[3]96 Deep Well Plates are reusable. After use, rinse the plate with water then incubate in 0.4M HCl for 1 minute at room temperature. Wash the plate thoroughly with ddH_2O. The plate can be autoclaved after being washed.

During the procedure, always wear a lab coat, disposable gloves, and protective goggles.

Quick Protocol Diagram



Presto™ 96 Well Blood gDNA Kit Protocol

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

IMPORTANT BEFORE USE!

1. Add ddH_2O pH7.0-8.5 (see the bottle label for volume) to Proteinase K then vortex to ensure Proteinase K is completely dissolved. Check the box on the bottle. For extended periods, the ddH_2O and Proteinase K mixture should be stored at 4ºC. Use only fresh ddH_2O as ambient CO_2 can quickly cause acidification.

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.

3. 96 Deep Well Plates are reusable. After use, rinse the plate with water then incubate in 0.4M HCl for 1 minute at room temperature. Wash the plate thoroughly with ddH_2O. The plate can be autoclaved after being washed.

Additional Requirements

Centrifuge with microplate buckets, 70ºC oven or incubator

Centrifuge Protocol Procedure

1. Sample Preparation and Lysis

Transfer **20 µl of Proteinase K** to the bottom of each microtube in **Microtubes (Racked)**. Transfer **up to 200 µl of whole blood, plasma, serum, body fluids or up to 5 x 10^6 lymphocytes or cultured cells in 200 µl PBS** to the **Microtubes (Racked)**, being careful not to touch the rims of the **Microtubes (Racked)** with the pipette tips.

Note: If the sample volume is less than 200 µl, use PBS to adjust the volume to 200 µl.

Add **200 µl of GB Buffer** to each sample, being careful not to touch the rims of the microtubes with the pipette tips. If RNA-free genomic DNA is required, add 5 µl of RNase A (50 mg/ml) to each sample. Seal the microtubes with the **Microtube Caps (8-strip)**. Cover the rack with the plastic lid then mix the sample thoroughly by shaking vigorously for 15 seconds. Incubate the **Microtubes (Racked)** at 70ºC in an oven or incubator for at least 10 minutes.

NOTE: At this time, pre-heat the required **Elution Buffer (400 µl per sample)** to 60ºC (for Step 4 DNA Elution).

2. DNA Binding

Briefly centrifuge the **Microtubes (Racked)** at 2,000 x g to collect any lysate from the caps. Allow the centrifuge to reach 2,000 x g prior to stopping. Remove the caps and add **200 µl of absolute ethanol** to each sample. Seal the **Microtubes (Racked)** with new caps. Cover the rack with the plastic lid then shake vigorously for 15 seconds. Briefly centrifuge the **Microtubes (Racked)** at 2,000 x g to collect any lysate from the caps. Allow the centrifuge to reach 2,000 x g prior to stopping. Place a **Presto™ gDNA 96 Well Binding Plate** on a **96 Deep Well Plate**. Remove the caps from the **Microtubes (Racked)** then transfer the lysate to each well of the **Presto™ gDNA 96 Well Binding Plate**, being careful not to get any lysate on the the rims of the wells. Centrifuge the **Presto™ gDNA 96 Well Binding Plate** and **96 Deep Well Plate** together at 3,000 x g for 5 minutes. Discard the flow-through. Place the **Presto™ gDNA 96 Well Binding Plate** back on the **96 Deep Well Plate**.

3. Wash

Add **400 µl of W1 Buffer** to each well of the **Presto™ gDNA 96 Well Binding Plate** then centrifuge the **Presto™ gDNA 96 Well Binding Plate** and **96 Deep Well Plate** together at 3,000 x g for 5 minutes. Discard the flow-through. Place the **Presto™ gDNA 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™ gDNA 96 Well Binding Plate**. Centrifuge the **Presto™ gDNA 96 Well Binding Plate** and **96 Deep Well Plate** together at 3,000 x g for 5 minutes. Discard the flow-through. Place the **Presto™ gDNA 96 Well Binding Plate** back on the **96 Deep Well Plate**. Centrifuge the **Presto™ gDNA 96 Well Binding Plate** and **96 Deep Well Plate** together at 3,000 x g for 10 minutes to dry the membrane.

4. Elution

Remove the **Presto™ gDNA 96 Well Binding Plate** from the **96 Deep Well Plate** then blot the nozzles on a clean absorbent paper towel to remove residual ethanol. Place the **Presto™ gDNA 96 Well Binding Plate** on **Microtubes (Racked)**. Add **200 µl of pre-heated Elution Buffer**[1], TE[2] or water[3] to the center of each well of the **Presto™ gDNA 96 Well Binding Plate**. Let stand for at least 2 minutes to ensure the Elution Buffer, TE or water is absorbed by the membrane. Centrifuge the **Presto™ gDNA 96 Well Binding Plate** and **Microtubes (Racked)** together at 3,000 x g for 5 minutes to elute the purified DNA. Seal the **Microtubes (Racked)** with new caps and store the purified DNA at -20ºC.

NOTE: For maximum DNA yield, repeat the elution step by adding 200 µl of pre-heated Elution Buffer, TE or water to each well of the Presto™ gDNA 96 Well Binding Plate then centrifuge again.

[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.

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™ gDNA 96 Well Binding Plate** in the binding top plate aperture. Attach the vacuum manifold to a vacuum source.

2. Sample Preparation and Lysis

Transfer **20 µl of Proteinase K** to the bottom of each microtube in a **Microtubes (Racked)**. Transfer **up to 200 µl of whole blood, plasma, serum, body fluids or up to 5 x 10^6 lymphocytes or cultured cells in 200 µl PBS** to the **Microtubes (Racked)**, being careful not to touch the rims of the **Microtubes (Racked)** with the pipette tips.

NOTE: If the sample volume is less than 200 µl, use PBS to adjust the volume to 200 µl.

Add **200 µl of GB Buffer** to each sample, being careful not to touch the rims of the microtubes with the pipette tips. If RNA-free genomic DNA is required, add 5 µl of RNase A (50 mg/ml) to each sample. Mix the samples by pipetting up and down 5 times then seal the microtubes with the **Microtube Caps (8-strip)**. Incubate the **Microtubes (Racked)** at 70ºC in an oven or incubator for at least 10 minutes.

Note: At this time, pre-heat the required **Elution Buffer (400 µl per sample)** to 60ºC (for Step 5 DNA Elution).

3. DNA Binding

Remove the caps and add **200 µl of absolute ethanol** to each sample then mix well by pipetting up and down 5 times. Transfer the lysate to each well of the **Presto™ gDNA 96 Well Binding Plate**, being careful not to get any lysate on the the rims of the wells.

NOTE: Seal unused wells of the Presto™ gDNA 96 Well Binding Plate with **Adhesive Film**.

Apply vacuum at 15 inches Hg until the samples pass through the **Presto™ gDNA 96 Well Binding Plate** then turn off the vacuum.

4. Wash

Add **400 µl of W1 Buffer** to each well of the **Presto™ gDNA 96 Well Binding Plate**. Apply vacuum at 15 inches Hg until **W1 Buffer** passes through the **Presto™ gDNA 96 Well Binding Plate** (approximately 10 seconds) then turn off the vacuum. Add **600 µl of Wash Buffer (make sure ethanol was added)** to each well of the **Presto™ gDNA 96 Well Binding Plate**. Apply vacuum at 15 inches Hg until **Wash Buffer** passes through the **Presto™ gDNA 96 Well Binding Plate**. Continue to apply vacuum for an additional 10 minutes to dry the membrane then turn off the vacuum.

5. Elution

Remove the **Presto™ gDNA 96 Well Binding Plate** from the manifold and blot the nozzles on clean, absorbent paper towel to remove residual ethanol. Remove the waste tray from the manifold base then place **Microtubes (Racked)** on the manifold base. Place the binding top plate on the manifold base then place the **Presto™ gDNA 96 Well Binding Plate** in the binding top plate aperture. Add **200 µl of pre-heated Elution Buffer[1], TE[2] or water[3]** to the **CENTER** of each well of the **Presto™ gDNA 96 Well Binding Plate**. Let stand for at least 2 minutes to ensure the Elution Buffer, TE or water is absorbed by the membrane. Apply vacuum at 15 inches Hg for 5 minutes to elute the purified DNA then turn off the vacuum. Seal the **Microtubes (Racked)** with new caps and store the purified DNA at -20ºC.

NOTE: For maximum DNA yield, repeat the elution step by adding 200 µl of pre-heated Elution Buffer, TE or water to each well of the Presto™ gDNA 96 Well Binding Plate then apply vacuum at 15 inches Hg for 5 minutes again.

[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 Blood Genomic DNA Kit Functional Test Data

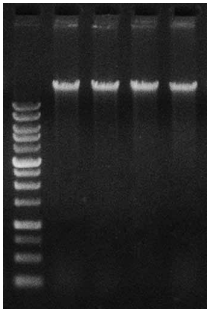


Figure 1. Genomic DNA was extracted from 200 µl whole human blood samples using the Presto™ 96 Well Blood Genomic DNA Extraction Kit. The purified genomic DNA was eluted in 200 µl of Elution Buffer and 15 µl aliquots of the final sample (chosen from 4 random wells) were analyzed by electrophoresis on a 1% agarose gel.
M = Geneaid™ 1 Kb DNA Ladder

Sample	ng/µl	260/280	Yield
1. 200 µl blood	27.6	1.81	4.7 µg
2. 200 µl blood	28.2	1.75	4.8 µg
3. 200 µl blood	32.9	1.74	5.6 µg
4. 200 µl blood	25.9	1.81	4.4 µg

Troubleshooting

Low Yield

DNA degradation due to improper storage of blood samples.

Yield and quality of DNA will be higher when fresh blood is used. Whole blood samples in anticoagulant treated tubes can be stored for several weeks at 4ºC. However, frozen blood can also be used. Increased storage length decreases DNA yield.

Incomplete 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.

Incomplete sample preparation.

After adding Proteinase K, samples and GB Buffer into microtubes, mix samples thoroughly by shaking vigorously or pipetting. DO NOT add Proteinase K directly to GB Buffer.

Clogged column.

Use the recommended amount of starting material. Overloading the columns will cause clogging and low DNA yield.

Incorrect DNA elution step.

Ensure that Elution Buffer, TE or water is added into the **CENTER** of the matrix and is completely absorbed. Use pre-heated Elution Buffer, TE, or water (60ºC). If using water for elution, ensure the water pH is between 7.5 and 8.5. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification. Elute twice to increase the DNA recovery.

Eluted DNA Does Not Perform Well In Downstream Applications

Residual ethanol contamination.

Following the wash step, dry the binding plate with additional centrifugation at 3,000 x g or with additional vacuum for 10 minutes to ensure the membrane is completely dry.

Residual RNA Contamination.

Perform the optional RNA removal step during sample preparation and lysis.

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
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
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 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	RBY050/100/300
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
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	DL004
1 Kb DNA Ladder	50 µg, 500 µl	DL006
Loading Dye (6X)	10/100 ml	LD010/100

Related DNA/RNA Extraction Products

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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