Geneaid

Instruction Manual
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For Research Use Only

Presto™ 96 Well Plant Genomic DNA Extraction Kit

96GPP02 (2 x 96 well plates/kit)
96GPP04 (4 x 96 well plates/kit)
96GPP10 (10 x 96 well plates/kit)

Advantages

Sample: up to 100 mg of fresh plant tissue and up to 25 mg of dry plant tissue per well
Yield: up to 30 µg of genomic DNA from 100 mg of fresh plant tissue per well
Format: Presto™ gDNA 96 Well Binding Plate
Operation Time: 60 minutes
Elution Volume: 200~400 µl
Kit Storage: dry at room temperature (15-25°C)

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Introduction

The Presto™ 96 Well Plant Genomic DNA Extraction Kit is designed for high-throughput purification of total DNA (including genomic DNA, mitochondrial and chloroplast DNA) from various plant species. Homogenized samples are treated with RNase A then centrifuged to remove cell debris and salt precipitates. In the presence of a binding buffer, genomic DNA in the lysate binds 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 entire procedure can be completed within 60 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 Plant Genomic DNA Extraction Kit is tested on a lot-to-lot basis by purifying genomic DNA from corn leaf samples. The purified DNA is quantified with a spectrophotometer and analyzed by electrophoresis.

Kit Components

Component	96GPP02	96GPP04	96GPP10
GP1 Buffer	100 ml	200 ml	500 ml
GPX1 Buffer	100 ml	200 ml	500 ml
GP2 Buffer	25 ml	50 ml	125 ml
GP3 Buffer[1] (Add Isopropanol)	45 ml (90 ml)	85 ml (170 ml)	45 ml x 1 (90 ml x 1) 85 ml x 2 (170 ml x 2)
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
RNase A (10 mg/ml)	1 ml	2 ml	5 ml
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

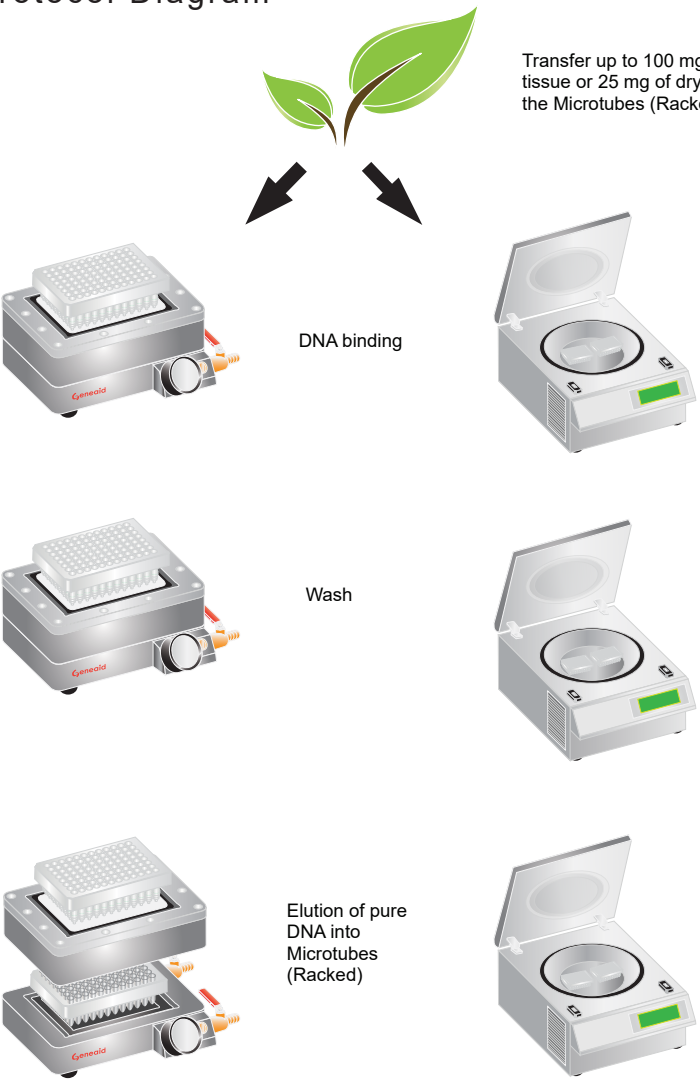
[1]Add Isopropanol (see the bottle label for volume) to GP3 Buffer then mix by shaking for a few seconds. Check the box on the bottle.

[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 Plant gDNA Kit Protocol

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

IMPORTANT BEFORE USE!

1. Add Isopropanol (see the bottle label for volume) to GP3 Buffer then mix by shaking for a few seconds. Check the box on the bottle.

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 or vacuum manifold, 60ºC incubator, 3 mm steal or ceramic beads, TissueLyser or mixer mill, additional 96 Deep Well Plate

Centrifuge Protocol Procedure

1. Sample Preparation

Various plant species contain different metabolites such as polysaccharides, polyphenols, and proteins. The standard protocol uses GP1 Buffer for lysis of most common plant species. The GP1 Buffer system ensures purified DNA with high yields and high quality. Alternatively, GPX1 Buffer is provided with the kit to ensure efficient cell lysis of plant species with high polysaccharide content.

Transfer up to 100 mg of fresh plant tissue or 25 mg of dry plant tissue into each tube of **Microtubes (Racked)**. Add a 3 mm steal bead or a ceramic bead to each tube. Seal the tubes with **Caps for Microtubes** then cover. Freeze the samples in liquid nitrogen for a few seconds. Using a TissueLyser or mixer mill, homogenize the samples by shaking at 30 Hz for 60-90 seconds or until a homogenous plant powder has been formed. If necessary, repeat shaking x 1. Centrifuge the samples at 4,000 x g for 2 minutes then remove caps.

NOTE: Fresh plant samples can also be homogenized in GP1 or GPX1 Buffer directly without liquid nitrogen. In this case, GP1 Buffer (or GPX1 Buffer) and RNase A can be added to each sample prior to homogenization. However, homogenization of fresh plants with lysis buffer may cause shearing of DNA.

2. Cell Lysis

Add **400 µl of GP1 Buffer or GPX1 Buffer** (for plant species with high polysaccharide content) and **5 µl of RNase A** to each sample. Seal the tubes with new **Caps for Microtubes** then mix by shaking vigorously for 15-30 seconds. Briefly centrifuge the **Microtubes (Racked)** at 1,500 x g for 30 seconds to collect any lysate from the caps. Incubate the samples at 60ºC for 20 minutes. During incubation, invert the samples every 5 minutes.

NOTE: Pre-heat the required volume of Elution Buffer (400 µl/ sample) to 60ºC for step 5 DNA elution.

Carefully open tubes then add **100 µl of GP2 Buffer** to each sample. Seal the tubes with caps. Mix the samples by shaking vigorously then incubate the samples on ice for 5 minutes. Centrifuge the samples at 5,000-6,000 x g for 15 minutes to clarify the sample lysate. Remove caps then transfer **400 µl of cleared lysate** to each well of a **96 Deep Well Plate**.

3. DNA Binding

Add **600 µl of GP3 Buffer (make sure isopropanol was added)** into each well of the **96 Deep well Plate** containing the sample lysate then mix well by pipetting up and down at least 3 times. Place the **Presto™ gDNA 96 Well Binding Plate** on a new **96 Deep Well Plate**. Transfer 500 µl of the sample lysate to each well of the **Presto™ gDNA 96 Well Binding Plate**, being careful not to get any lysate on 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. Transfer the remaining sample lysate to each well of the **Presto™ gDNA 96 Well Binding Plate**, being careful not to get any lysate on 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 then place the **Presto™ gDNA 96 Well Binding Plate** back on the **96 Deep Well Plate**.

4. Wash

Add **400 µl of W1 Buffer** to each well of the **Presto™ gDNA 96 Well Binding Plate** then centrifuge with the **96 Deep Well Plate** at 3,000 x g for 5 minutes. Discard the flow-through then 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** then centrifuge with the **96 Deep Well Plate** at 3,000 x g for 5 minutes. Discard the flow-through then 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.

Optional Step: Residual Pigment Removal (If pigments remain on the column matrix, perform this step)

Following Wash Buffer addition, add 400 µl of absolute ethanol to each well of the Presto™ gDNA 96 Well Binding Plate then centrifuge with the 96 Deep Well Plate at 3,000 x g for 5 minutes. Discard the flow-through. 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.

5. 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

Various plant species contain different metabolites such as polysaccharides, polyphenols, and proteins. The standard protocol uses GP1 Buffer for lysis of most common plant species. The GP1 Buffer system ensures purified DNA with high yields and high quality. Alternatively, GPX1 Buffer is provided with the kit to ensure efficient cell lysis of plant species with high polysaccharide content.

Transfer up to 100 mg of fresh plant tissue or 25 mg of dry plant tissue into each tube of **Microtubes (Racked)**. Add a 3 mm steal bead or a ceramic bead to each tube. Seal the tubes with **Caps for Microtubes** then cover. Freeze the samples in liquid nitrogen for a few seconds. Using a TissueLyser or mixer mill, homogenize the samples by shaking at 30 Hz for 60-90 seconds or until a homogenous plant powder has been formed. If necessary, repeat shaking x 1. Centrifuge the samples at 4,000 x g for 2 minutes then remove caps.

NOTE: Fresh plant samples can also be homogenized in GP1 or GPX1 Buffer directly without liquid nitrogen. In this case, GP1 Buffer (or GPX1 Buffer) and RNase A can be added to each sample prior to homogenization. However, homogenization of fresh plants with lysis buffer may cause shearing of DNA.

3. Cell Lysis

Add **400 µl of GP1 Buffer or GPX1 Buffer** (for plant species with high polysaccharide content) and **5 µl of RNase A** to each sample. Seal the tubes with new **Caps for Microtubes** then mix by shaking vigorously for 15-30 seconds. Briefly centrifuge the **Microtubes (Racked)** at 1,500 x g for 30 seconds to collect any lysate from the caps. Incubate the samples at 60ºC for 20 minutes. During incubation, invert the samples every 5 minutes.

NOTE: Pre-heat the required volume of Elution Buffer (400 µl/ sample) to 60ºC for step 6 DNA elution.

Carefully open tubes and add **100 µl of GP2 Buffer** to each sample. Seal the tubes with caps. Mix the samples by shaking vigorously then incubate the samples on ice for 5 minutes. Centrifuge the samples at 5,000-6,000 x g for 15 minutes to clarify the sample lysate. Remove caps then transfer **400 µl of cleared lysate** to each well of a **96 Deep Well Plate**.

4. DNA Binding

Add **600 µl of GP3 Buffer (make sure isopropanol was added)** into each well of the **96 Deep Well Plate** containing the sample lysate then mix well by pipetting up and down at least 3 times. Transfer all of the sample lysate (approx. 1 ml) to each well of the **Presto™ gDNA 96 Well Binding Plate**, being careful not to get any lysate on 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 switch off the vacuum.

5. 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** (approx. 10 seconds) then switch 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.

Optional step: Residual Pigment Removal (If pigments remain on the column matrix, perform this step)

Following Wash Buffer addition, add 400 µl of absolute ethanol to each well of the Presto™ gDNA 96 Well Binding Plate. Apply vacuum at 15 inches Hg until ethanol 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.

6. 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 Plant Genomic DNA Kit Functional Test Data

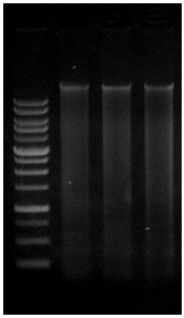


Figure 1. Genomic DNA was extracted from 50 mg of fresh corn leaf samples using the Presto™ 96 Well Plant Genomic DNA Extraction Kit. The purified genomic DNA was eluted in 200 µl of Elution Buffer and 15 µl aliquots of the final sample (chosen from 3 random wells) were analyzed by electrophoresis on a 1% agarose gel.
M = Geneaid™ 1 Kb DNA Ladder

Sample	DNA Conc.	260/280	260/230	Yield
1	77.7 ng/µl	1.80	2.02	13.6 µg
2	78.8 ng/µl	1.80	2.05	13.8 µg
3	76 ng/µl	1.79	1.99	13.3 µg

Troubleshooting

Low Yield

Inappropriate lysis buffer was used

Two lysis buffers (GP1 and GPX1) are offered with this kit. GP1 Buffer is suitable for lysis of most common plant species and GPX1 Buffer is used for efficient lysis of plant cells with high polysaccharide content. In order to ensure the optimal lysis buffer is being used, we recommend trying both lysis buffers for sample homogenization.

Incomplete buffer preparation.

Add isopropanol (see the bottle label for volume) to GP3 Buffer and absolute ethanol (see the bottle label for volume) to Wash Buffer prior to use. Be sure and close the bottle tightly after each use to avoid evaporation.

Plant samples are too old.

Yield and quality of DNA will be higher when fresh plant tissue is used. If plant samples must be stored for long periods, freeze the samples at -20 to -80ºC.

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.

Add 5 µl of provided RNase A into each sample lysate to remove RNA.

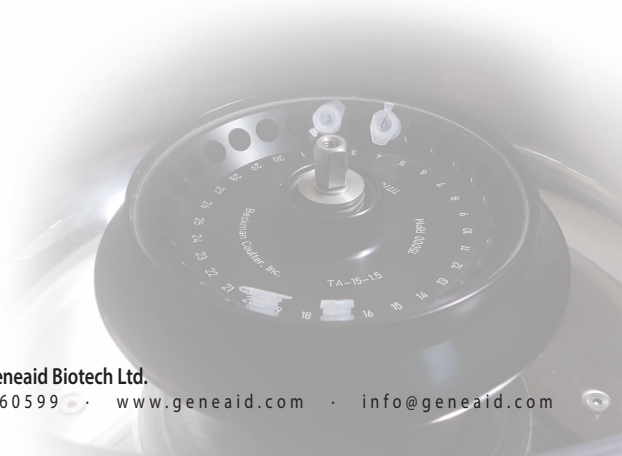
Related DNA/RNA Extraction Products

Plasmid DNA Purification		
Product	Package Size	Catalogue Number
Presto™ Mini Plasmid Kit	100/300 preps	PDH100/300
Presto™ Endotoxin Free Mini Plasmid Kit	100 preps	PEH100
Presto™ Midi Plasmid Kit	25 preps	PIF025
Presto™ Midi Plasmid Kit (Endotoxin Free)	25 preps	PIFE25
Large Plasmid DNA Extraction Kit	100/300 preps	PDL100/300
Midiprep Spin Column Plasmid Kit	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
Large DNA Fragments Extraction Kit	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		
Product	Package Size	Catalogue Number
Genomic DNA Mini Kit (Blood/Cultured Cell)	100/300 preps	GB100/300
Genomic DNA Maxi Kit (Blood/Cultured Cell)	10/25 preps	GDM010/25
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	GPM010/25
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
Presto™ 96 Well Plant Genomic DNA Extraction Kit	4/10 x 96 preps	96GPP04/10
DNA RNA Purification		
Product	Package Size	Catalogue Number
Presto™ DNA/RNA Extraction Kit	50/100 preps	DR050/100
Presto™ DNA/RNA/Protein Extraction Kit	50/100 preps	DRP050/100

Related DNA/RNA Extraction Products

RNA 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
GENEzol™ 96 Well TriRNA Pure Kit	4/10 x 96 preps	96GZX04/10
TriRNA Pure Kit	50/100/200 preps	TRP050/100/200
RNA Cleanup 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
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

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



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UKAS QUALITY MANAGEMENT

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