Geneaid

Instruction Manual
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For Research Use Only

Presto™ DNA/RNA/Protein Extraction Kit

DRP004 (4 Preparation Sample Kit)
DRP050 (50 Preparation Kit)
DRP100 (100 Preparation Kit)

Advantages

Sample: cultured animal cells (up to 5 x 10^6), up to 25 mg of tissue, up to 500 µl of whole human blood, up to 200 µl of biological liquids (serum, plasma)
Yield: up to 9 µg of genomic DNA, 20 µg of total RNA, 120 µg of protein from 1.5 x 10^6 HeLa cells
Format: genomic DNA spin column and total RNA spin column
Operation Time: DNA/RNA purification within 25 minutes, protein precipitation within 50 minutes
Elution Volume: 50-200 µl (genomic DNA) / 25-50 µl (total RNA)
Kit Storage: dry at room temperature (15-25°C)

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Introduction

The Presto™ DNA/RNA/Protein Extraction Kit provides an efficient method for purifying genomic DNA, total RNA and total protein simultaneously from a variety of samples (cultured cells, animal tissue, whole blood and biological liquids). Chaotropic salt is used to lyse cells and inactive DNases and RNases, allowing DNA to bind to the genomic DNA spin column. The flow-through can then be transferred to the RNA spin column for RNA binding. The proteins in the flow-through can then be precipitated using acetone. Contaminants are effectively removed using wash buffers followed by pure genomic DNA elution in a low salt buffer and pure total RNA elution in RNase-free Water. DNA/RNA purification can be completed in 25 minutes without phenol/chloroform extraction or alcohol precipitation and protein purification can be completed in 50 minutes. The purified DNA, with approximately 20-30 Kb, is suitable for use in PCR or other enzymatic reactions and the purified RNA (including miRNA) is ready for use in RT-PCR, Real-time PCR, northern blotting, primer extension, mRNA selection and cDNA synthesis. The purified proteins can be directly analyzed on a SDS-PAGE and subsequent western blot.

Quality Control

The quality of the Presto™ DNA/RNA/Protein Extraction Kit is tested on a lot-to-lot basis by isolating genomic DNA, total RNA and protein from cultured animal cells. The purified DNA and total RNA is quantified with a spectrophotometer and analyzed by electrophoresis on a 1% agarose gel. The purified protein is quantified by bradford assay and analyzed on SDS-PAGE.

Kit Components

Component	DRP004	DRP050	DRP100
RBC Lysis Buffer	10 ml	100 ml	200 ml
DR Buffer	2 ml	30 ml	60 ml
RW1 Buffer	2 ml	30 ml	50 ml
RPE Buffer[1] (Add Ethanol)	1 ml (4 ml)	25 ml (100 ml)	50 ml (200 ml)
W1 Buffer	2 ml	45 ml	45 ml
Wash Buffer[1] (Add Ethanol)	1 ml (4 ml)	12.5 ml (50 ml)	25 ml (100 ml)
RNase-free Water	1 ml	6 ml	6 ml
Elution Buffer	1 ml	30 ml	30 ml
DV Buffer (8M Urea)	500 µl	6 ml	12 ml
Protein Loading Dye	60 µl	2 ml	2 ml
RB Column	4	50	100
GD Column	4	50	100
2 ml Collection Tube	16	200	400

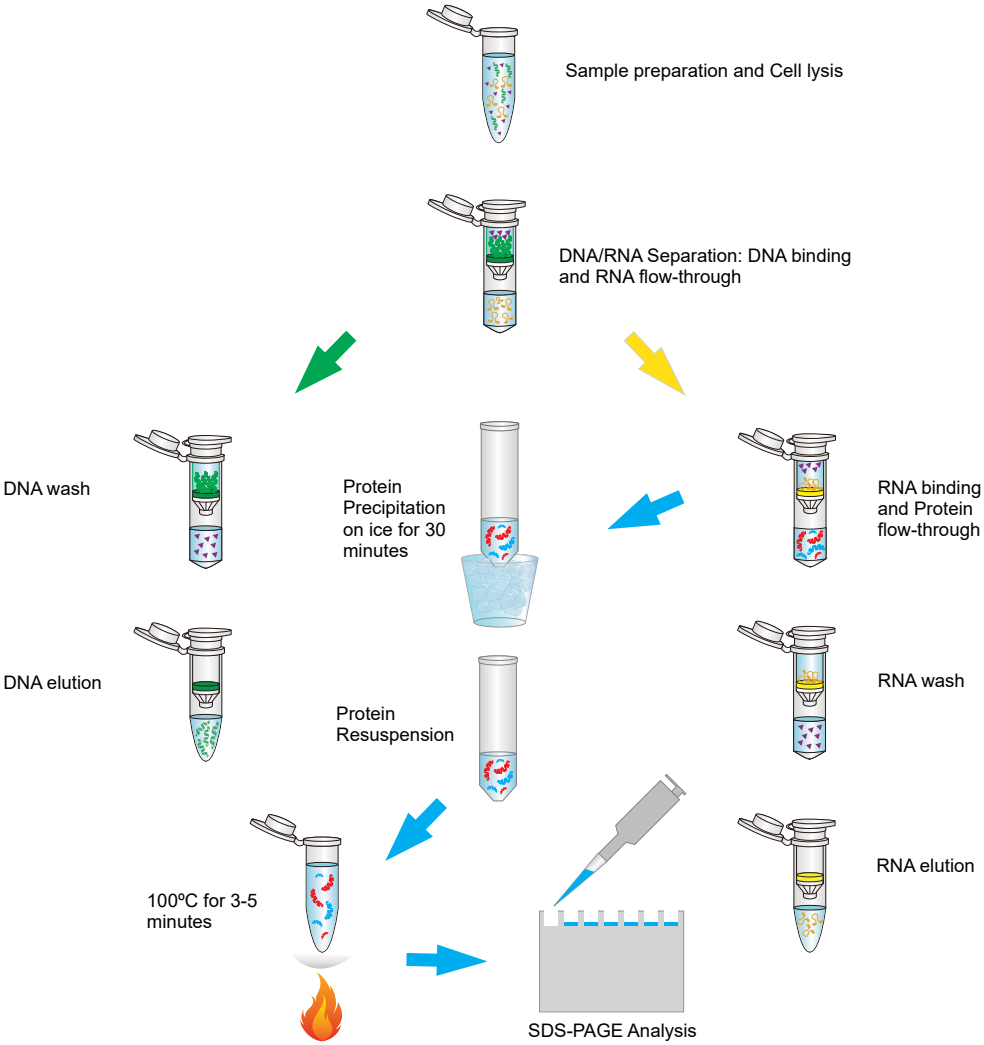
[1]Add absolute ethanol (see the bottle label for volume) to RPE Buffer and Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

Steps to prevent RNase contamination

1. Always wear a lab coat, disposable gloves, protective goggles and (anti-fog) procedure mask.
2. Plasticware and automatic pipettes should be sterile (RNase-free) and used only for RNA procedures.
3. Non-disposable glassware or plasticware should also be sterile (RNase-free).

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

Quick Protocol Diagram



Presto™ DNA/RNA/Protein 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 RPE Buffer and 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. Prepare Phosphate Buffered Saline (PBS, pH7.2) for adherent cultured cells.

3. Yield and quality of DNA/RNA will be higher when fresh samples or samples which have been flash frozen and stored at -70ºC are used. DNA/RNA in samples which has been repeatedly frozen and thawed may be degraded.

Additional Requirements

absolute ethanol, ß-mercaptoethanol, acetone; for cell samples: phosphate-buffered saline (PBS), 0.10-0.25% Trypsin; for tissue samples: TissueLyser or mortar and pestle, 20-G needle syringe

NOTE: Dithiothreitol (DTT) can be used as an alternative reducing agent in DR Buffer instead of ß-mercaptoethanol. Immediately prior to use, add 40 mM DTT per reaction to DR Buffer. For example, add 20 µl of 2 M DTT in RNase-free Water to 1 ml of DR Buffer then mix well.

Protocol Procedure

1. Sample Preparation

Adherent Cultured Animal Cells

A. Cell lysis in a culture dish

Aspirate the culture medium completely. **Add 400 µl of DR Buffer and 4 µl of ß-mercaptoethanol** immediately to the culture dish (up to 5 x10^6 cells). Incubate at room temperature for 5 minutes then transfer the cell lysate to a 1.5 ml microcentrifuge tube.

B. Trypsinize cell prior to cell lysis

Remove the culture medium and wash cells in PBS. Aspirate PBS and add 0.10-0.25% Trypsin in PBS. Once cells have detached, add the medium and transfer to a 15 ml centrifuge tube. Proceed with Suspension Cultured Animal Cells.

Suspension Cultured Animal Cells

Transfer cells (up to 5 x 10^6) to a 1.5 ml microcentrifuge tube or 15 ml centrifuge tube then centrifuge for 5 minutes at 300 x g. Remove the supernatant completely then add **400 µl of DR Buffer** and **4 µl of ß-mercaptoethanol**. Resuspend cells by pipette/vortex. Proceed with DNA/RNA Separation.

Animal Tissue

Excise 10-25 mg of tissue from the animal or remove tissue sample from storage. Do not use more than 25 mg of tissue per reaction. Homogenize tissue using one of the following methods: A. Transfer tissue to a 2 ml centrifuge tube containing ceramic beads or stainless steel beads, add **400 µl of DR Buffer** and **4 µl of ß-mercaptoethanol** to the tissue sample and then homogenize the sample with a TissueLyser, Disruptor Genie or similar. B. Freeze the tissue in liquid nitrogen then grind the tissue thoroughly with a mortar and pestle. Transfer the tissue powder to a 1.5 ml microcentrifuge tube (do not allow the tissue to thaw) and add **400 µl of DR Buffer** and **4 µl of ß-mercaptoethanol**. Shear the tissue by passing the lysate through a 20-G needle syringe 10 times. Proceed with Step 2 DNA/RNA Separation.

Biological Fluids

Add **300 µl of DR Buffer** and **3 µl of ß-mercaptoethanol** to 100 µl of liquid sample and mix well by pipetting or vortex. Proceed with Step 2 DNA/RNA Separation.

Human Whole Blood

Collect fresh human blood in anticoagulant-treated collection tubes. Transfer 500 µl of blood to a sterile 15 ml centrifuge tube. Add **1.5 ml of RBC Lysis Buffer (3 volumes)** and mix by inversion. Incubate the tube on ice for 10 minutes (vortex twice briefly during incubation). Centrifuge at 3,000 x g for 5 minutes then remove the supernatant completely. Add **400 µl of DR Buffer** and **4 µl of ß-mercaptoethanol** to resuspend leukocyte pellet by pipetting or vortex. Proceed with Step 2 DNA/RNA Separation.

2. DNA/RNA Separation

Incubate the sample lysate at room temperature for 5 minutes then centrifuge at 12-16,000 x g for 2 minutes. Place a **GD Column** in a 2 ml Collection Tube then transfer the supernatant to the **GD Column**. Centrifuge at 14-16,000 x g for 1 minute. Note: If the lysate mixture could not flow past the GD Column membrane following centrifugation, increase the centrifuge time until it passes completely. Save the flow-through in the 2 ml Collection Tube for RNA Purification. Place the **GD Column** in a new 2 ml Collection Tube and store at room temperature (15-25ºC) or 4ºC for DNA Purification. Do not store the GD Column for extended periods. Do not freeze the GD Column. At this time, preheat the required Elution Buffer (200 µl per sample) to 60ºC (for DNA elution).

RNA Purification

3. RNA Binding

Add **0.8 volume of absolute ethanol** to the flow-through in the 2 ml Collection Tube (e.g. add 320 µl of absolute ethanol to 400 µl of flow-through) and mix well by pipetting. Transfer the sample to the **RB Column in a 2 ml Collection Tube**. Centrifuge at 14-16,000 x g for 1 minute. Place the **RB Column** in a new 2 ml Collection Tube and save the flow-through for protein purification. Note: If DNA-free RNA is required, perform optional In Column DNase I Digestion below.

Optional In Column DNase I Digestion

Add **400 µl of RPE Buffer (make sure ethanol was added)** to the **RB Column** then centrifuge at 14-16,000 x g for 30 seconds. Discard the flow-through and place the **RB Column** back in the 2 ml Collection Tube. Prepare DNase I solution in a 1.5 ml microcentrifuge tube (RNase-free) as follows:

DNase I	5 µl (2 U/µl)
DNase I Reaction Buffer	45 µl
Total Volume	50 µl

RNase-Free DNase I set can be purchased directly from Geneaid (cat. # DNS050/100/300). Standard DNase buffers are incompatible with this in-column DNase digestion, which will effect RNA integrity and reduce yield.

Mix DNase I solution by pipetting gently (DO NOT vortex). Add **DNase I solution (50 µl)** into the CENTER of the **RB column** matrix. Incubate the column for 15 minutes at room temperature (20-30ºC) then proceed with RNA Wash on page 6.

4. RNA Wash

Add **400 µl of RW1 Buffer** into the **RB Column** and centrifuge at 14-16,000 x g for 30 seconds. Discard the flow-through and place the **RB Column** back in the 2 ml Collection Tube. Add **600 µl of RPE Buffer (make sure ethanol was added)** into the **RB Column**. Centrifuge at 14-16,000 x g for 30 seconds. Discard the flow-through and place the **RB Column** back in the 2 ml Collection Tube. **Add 600 µl of RPE Buffer (make sure ethanol was added)** into the **RB Column**. Centrifuge at 14-16,000 x g for 30 seconds. Discard the flow-through and place the **RB Column** back in the 2 ml Collection Tube. Centrifuge at 14-16,000 x g for 3 minutes to dry the column matrix.

5. RNA Elution

Place the **RB Column** in a clean 1.5 ml microcentrifuge tube (RNase-free). Add **25-50 µl of RNase-free Water** into the **CENTER** of the column matrix. Let stand for at least 1 minute to ensure the RNase-free Water is absorbed by the matrix. Centrifuge at 14-16,000 x g for 1 minute to elute the purified RNA.

Protein Purification

6. Protein Precipitation

Add **4 volumes of ice-cold acetone** to the flow-through from step 3 in a 15 ml centrifuge tube (example: add 2.8 ml of ice-cold acetone to 700 µl flow-through). Incubate on ice or at -20ºC for 30 minutes. At this time, perform DNA Purification on page 7. Centrifuge at 14-16,000 x g for 10 minutes then discard the supernatant.

7. Protein Resuspension

Add **100 µl of ice-cold 70% ethanol** to wash the protein pellet. Discard the supernatant then air-dry the protein pellet at room temperature.
Note: DO NOT overdry the protein pellet as it may results in difficult resuspension.
Add up to 100 µl of DV buffer (8M urea) or a buffer compatible with downstream application to dissolve the protein pellet.

8. SDS-PAGE Analysis

Add **2 µl of Protein Loading Dye** and 0.5 µl of 2M DTT (optional) into a clean microcentrifuge tube. Add **8 µl of the protein sample** then mix well by pipetting. Incubate protein sample at 100ºC for 3-5 minutes. Centrifuge the protein sample briefly then directly load to SDS-PAGE.

DNA Purification

9. DNA Wash

Add **400 µl of W1 Buffer** to the **GD Column**. Centrifuge at 14-16,000 x g for 30 seconds then discard the flow-through. Place the **GD Column** back in the 2 ml Collection Tube. Add **600 µl of Wash Buffer (make sure absolute ethanol was added)** to the **GD Column**. Centrifuge at 14-16,000 x g for 30 seconds then discard the flow-through. Place the **GD Column** back in the 2 ml Collection Tube. Centrifuge again for 3 minutes at 14-16,000 x g to dry the column matrix.

NOTE: Additional centrifugation at 14-16,000 x g for 5 minutes or incubation at 60ºC for 5 minutes will completely dry the GD Column to avoid any residual ethanol carryover and ensure the most effective downstream applications.

10. DNA Elution

Standard elution volume is 100 µl. If less sample is to be used, reduce the elution volume (30-50 µl) to increase DNA concentration. If higher DNA yield is required, repeat the DNA Elution step to increase DNA recovery and the total elution volume to approximately 200 µl.

Transfer the dried **GD Column** to a clean 1.5 ml microcentrifuge tube. Add **100 µl of pre-heated Elution Buffer**[1], TE Buffer[2] or water[3] into the **CENTER** of the column matrix. Let stand for at least 3 minutes to allow Elution Buffer, TE Buffer or water to be completely absorbed. Centrifuge at 14-16,000 x g for 30 seconds 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 GD 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 GD Column matrix and is completely absorbed.

[3]If using water for elution, ensure the water pH is between 7.0 and 8.5. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification. Ensure that water is added into the center of the GD Column matrix and is completely absorbed. DNA Eluted in water should be stored at -20ºC to avoid degradation.

Troubleshooting

Low Yield

Improper sample homogenization.

Yield and quality of DNA/RNA will be higher when fresh samples or samples which have been flash frozen and stored at -70ºC are used. DNA/RNA in samples which has been repeatedly frozen and thawed may be degraded. Fresh blood is recommended. However, frozen or blood treated with anticoagulants can also be used. Increased storage length decreases DNA/RNA yield. Overloading the columns causes low nucleic acid yield.

Incomplete buffer preparation.

Add absolute ethanol (see the bottle label for volume) to RPE Buffer and 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. Pre-heat Elution Buffer to 60ºC prior to DNA elution.

Incorrect elution step.

Ensure that Elution Buffer, TE or water is added into the **CENTER** of the GD Column matrix and is completely absorbed. Make sure RNase-free Water is added to the **CENTER** of the RB Column and is absorbed completely. Use pre-heated Elution Buffer, TE, or water (60~70ºC). If using water for elution, ensure the water pH is between 7.0 and 8.5. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification. Elute twice to increase the DNA/RNA recovery.

Clogged column.

Use the recommended amount of starting material or separate into multiple tubes. After homogenization, centrifuge the lysate at 16,000 x g for 5 minutes to precipitate insoluble cell debris, make sure only supernatant was transferred to GD Column. All centrifugation steps should be at room temperature (20-25ºC).

DNA Contaminated With RNA

Lysate applied to GD Column contains ethanol.

Only add the appropriate volume of ethanol to the lysate after passing lysate through the GD Column.

Low A260/280

Improper buffer preparation.

Add appropriate volume of absolute ethanol (see the bottle label) to the RPE Buffer and Wash Buffer prior to use.

Incorrect buffer used for nucleic acid dilution.

Use Elution Buffer (10 mM Tris, pH=8.0) instead of RNase-free Water for DNA/RNA dilution before measuring purity.

Degraded RNA

Incorrect sample preparation and/or storage.

Process or freeze samples at -70ºC immediately after collection.

Incorrect storage temperature.

Extracted RNA should be stored at -70ºC.

Eluted DNA/RNA Does Not Perform Well In Downstream Applications

Residual Ethanol Contamination.

Following the wash step, dry the GD/RB Column with additional centrifugation at 14-16,000 x g for 5 minutes to remove residual ethanol.

No Protein Detected On Western Blot Or Coomassie Stained Gel

Protein pellet loss.

At the protein precipitation step, the protein pellet is loosely attached to the side of the 15 ml centrifuge tube. Carefully decant the supernatant.

The Presto™ DNA/RNA/Protein Extraction Kit Functional Test Data

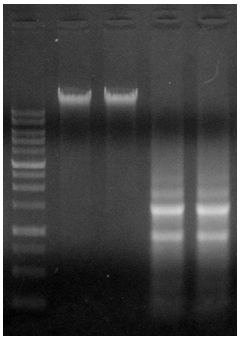


Figure 1. Genomic DNA and Total RNA from 1.5 x 10^6 HeLa cells was extracted using the Presto™ DNA/RNA/Protein Extraction Kit. 10 µl aliquots from a 200 µl eluate of purified genomic DNA and 10 µl aliquots from a 50 µl eluate of purified total RNA were analyzed by electrophoresis on a 1% agarose gel.
1-2 = DNA from 1.5 x 10^6 HeLa cells
3-4 = RNA from 1.5 x 10^6 HeLa cells
M = Geneaid 1 Kb DNA Ladder

Sample	µg/ml	260/280	260/230	Yield
1. DNA	42.7	1.88	2.19	8.54 µg
2. DNA	46.7	1.88	2.18	9.34 µg
3. RNA	417.5	2.09	2.03	20.90 µg
4. RNA	429.9	2.09	2.04	21.50 µg

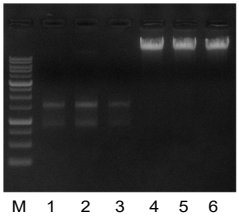


Figure 2. Genomic DNA and Total RNA from 3 human blood samples (500 µl) was extracted using the Presto™ DNA/RNA/Protein Extraction Kit. DNA yield: 8.0-10.0 µg (100 µl eluate, sample 4-6) RNA yield: 0.5-1.0 µg (50 µl eluate, sample 1-3). 10 µl aliquots from a 100 µl eluate of purified genomic DNA and 10 µl aliquots from a 50 µl eluate of purified total RNA were analyzed by electrophoresis on a 1% agarose gel.
M = Geneaid 1 Kb DNA Ladder

Sample	ng/µl	260/280	260/230	Yield (µg)
1. RNA	13.1	1.84	1.91	0.7
2. RNA	20.8	1.93	1.97	1.0
3. RNA	12.1	1.89	1.89	0.6
4. DNA	93.4	1.83	2.18	9.3
5. DNA	103.1	1.85	2.12	10.3
6. DNA	89.1	1.83	2.16	8.9

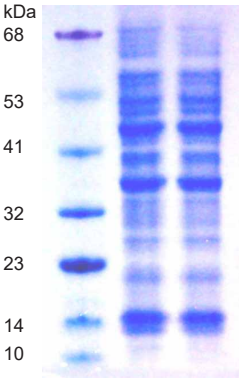


Figure 3. Protein was extracted from 1.5 x 10^6 HeLa Cells using the Presto™ DNA/RNA/Protein Extraction Kit. 20 µl of aliquots from a 200 µl eluate of purified protein were analyzed on a NuPAGE4-12% Bis-Tris gel and stained with coomassie blue.

Related DNA/RNA Extraction Products

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
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
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
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

Related DNA/RNA Extraction Products

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		
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	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
GENEzol™ DNA Reagent Plant	100/200 rxns	GR100/200
Presto™ Mini gDNA Yeast Kit	100/300 preps	GBYB100/300
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

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

Geneaid

INTERNATIONAL

UKAS QUALITY MANAGEMENT

Registration Number 046

CERTIFICATE NO. QAIC/TW/50077

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