Geneaid

Instruction Manual
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For Research Use Only

gSYNC™ 96 Well DNA Extraction Kit

96GS002 (2 x 96 Well Plates/Kit)
96GS004 (4 x 96 Well Plates/Kit)
96GS010 (10 x 96 Well Plates/Kit)

Advantages

Sample: tissue, rodent tails, ear punches and cultured cells
Yield: 10-20 µg of gDNA from 20 mg of tissue
Format: Presto™ gDNA 96 Well Binding Plates
Operation Time: within 60 minutes
Elution Volume: 100-400 µl
Kit Storage: dry at room temperature (15-25°C)

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Introduction

The gSYNC™ 96 Well DNA Extraction Kit is optimized for genomic, mitochondrial and virus DNA purification from tissue or 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 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 gSYNC™ 96 Well DNA Extraction Kit is tested on a lot-to-lot basis by isolating genomic DNA from tissue samples. The purified DNA is quantified with a spectrophotometer and analyzed by electrophoresis.

Kit Components

Component	96GS002	96GS004	96GS010
GST Buffer	60 ml	100 ml	200 ml
GSB Buffer	60 ml	100 ml	155 ml x 1 60 ml x 1
W1 Buffer	80 ml	200 ml	200 ml x 2
Wash Buffer[1] (Add Ethanol)	25 ml (100 ml)	50 ml (200 ml)	25 ml x 1 (100 ml) 50 ml x 2 (200 ml x 2)
Proteinase K[2] (Add ddH_2O)	11 mg x 4 (1.1 ml x 4)	65 mg x 1 (6.5 ml) 11 mg x 2 (1.1 ml x 2)	55 mg x 4 (5.5 ml x 4)
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

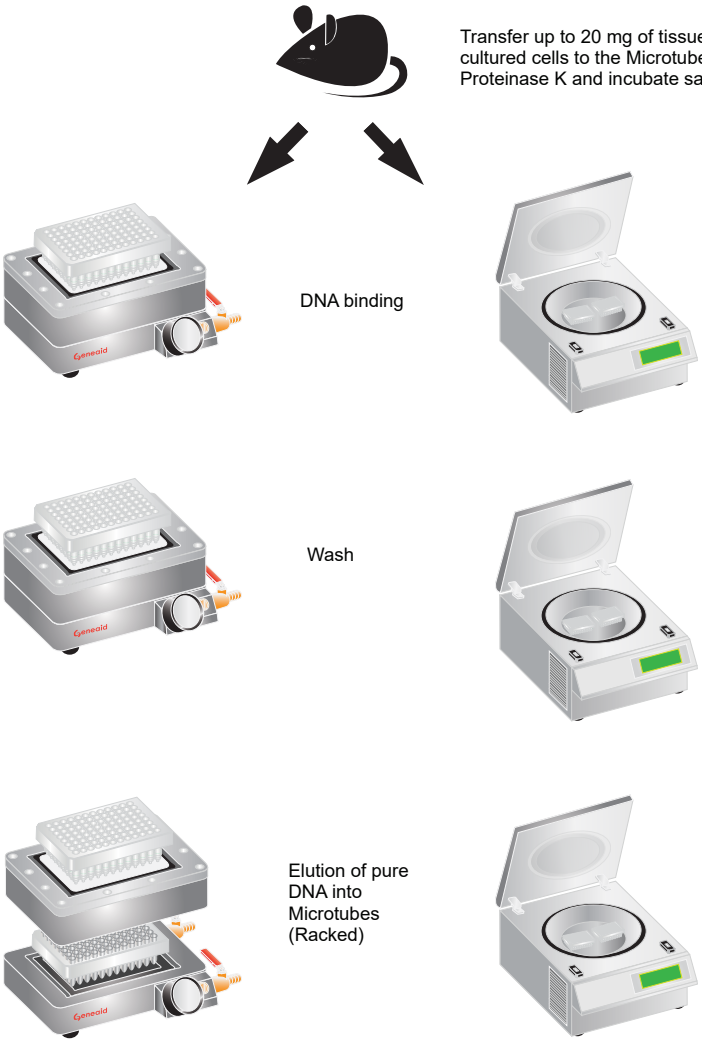
[1]Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

[2]Add ddH_2O to Proteinase K (see the bottle label for volume) then vortex to ensure it is completely dissolved. Check the box on the bottle. Once it is dissolved completely, centrifuge for a few seconds to spin the mixture down. 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.

[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



gSYNC™ 96 Well DNA 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. close the bottle tightly after each use to avoid ethanol evaporation.

2. Add ddH_2O pH7.0-8.5 (see the bottle label for volume) to Proteinase K then vortex to ensure it is completely dissolved. Check the box on the bottle. Once it is dissolved completely, centrifuge for a few seconds to spin down the mixture. 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.

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

4. Yield and quality of DNA will be higher when fresh samples or flash frozen (-20ºC or -70ºC) samples are used. DNA in tissue which has been repeatedly frozen and thawed may be degraded.

Proteinase K Working Solution Preparation

A. Mix **20 µl of Proteinase K and 180 µl of GST Buffer** per sample by vortex.

B. For 96 samples, mix **2 ml of Proteinase K and 18 ml of GST Buffer** by vortex.

Binding Solution Preparation

A. Mix **200 µl of GSB Buffer and 200 µl of absolute ethanol** per sample by shaking.

B. For 96 samples, mix **20 ml of GSB Buffer and 20 ml of absolute ethanol** by shaking.

Additional Requirements

centrifuge with microplate buckets or vacuum manifold, 60ºC incubator, additional 96 deep well plate, absolute ethanol, tube for per sample Proteinase K working solution and binding solution, 50 ml centrifuge tube for 96 sample Proteinase K working solution and binding solution

Centrifuge Protocol Procedure

1. Tissue Dissociation and Lysis

Transfer up to **20 mg of animal tissue (0.5 cm mouse tail) or 1 x 10^6 cultured cells** into each tube of **Microtubes (Racked)**. Add **200 µl of Proteinase K working solution** to each microtube. Seal the tubes with the **Caps for Microtubes (8-strip)** then cover the rack with the plastic cover and mix by inverting. Briefly centrifuge at 3,000 x g to collect any solution from the caps. Allow the centrifuge to reach 3,000 x g prior to stopping. It's important that the tissue samples are completely immersed in Proteinase K working solution. Incubate at 60ºC until the sample lysate becomes clear (at least 6 hours or 10 minutes for cultured cells). Place a weight on top of the plastic cover during incubation. For optimal lysis, mix occasionally or place the rack on a rocking platform during incubation. After incubation, make sure the microtubes are sealed properly. Cover the rack with the plastic cover then mix the sample lysate by shaking vigorously for 10 seconds. Briefly centrifuge at 3,000 x g to collect any solution from the caps. Allow the centrifuge to reach 3,000 x g prior to stopping.

Optional RNA removal step: For RNA-free gDNA, add 5 µl of RNase A (10 mg/ml) into each microtube. Seal the tubes with new caps (not provided). Cover the rack with the plastic cover then mix by shaking vigorously. Briefly centrifuge at 3,000 x g to collect any solution from the caps. Allow the centrifuge to reach 3,000 x g prior to stopping. Incubate for 5 minutes at room temperature.

NOTE: Tissue homogenization prior to incubation will facilitate Proteinase K digestion and cell lysis subsequently increasing DNA yield. Inverting the sample occasionally during incubation will also facilitate Proteinase K digestion and cell lysis. Using an auto shaking system is more convenient when incubating samples. During incubation, pre-heat the required volume of Elution Buffer (400 µl/sample) to 60ºC (for Step 4 DNA Elution).

2. DNA Binding

Carefully open **Microtubes (Racked)** and add **400 µl of Binding Solution** to each microtube. Seal the microtubes with new caps. Cover the rack with the plastic cover then mix by shaking vigorously for 15-30 seconds. Briefly centrifuge at 3,000 x g to collect any lysate from the caps. Allow the centrifuge to reach 3,000 x g prior to stopping. Place a **Presto™ gDNA 96 Well Binding Plate** on a new **96 Deep Well Plate**. Carefully open microtubes and transfer all of the sample lysate into each well of the **Presto™ gDNA 96 Well Binding Plate** being careful not to get any lysate on the rims of the wells. DO NOT transfer insoluble particles such as hair or bones to the **Presto™ gDNA 96 Well Binding Plate** to prevent membrane clogging. 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**.

3. Wash

Add **400 µl of W1 Buffer** to each well of the **Presto™ gDNA 96 Well Binding Plate** then centrifuge together 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 together with the **96 Deep Well Plate** 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** and centrifuge 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 top of **Microtubes (Racked)**. Add **200 µl of pre-heated Elution Buffer**[1], TE[2] or water[3] into 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 the **Microtubes (Racked)** together at 3,000 x g for 5 minutes. Seal the microtubes with new caps and store the purified DNA at -20ºC.

1. For the maximum DNA yield, repeat the elution step by adding another 200 µl of pre-heated Elution Buffer to each well of the Presto™ gDNA 96 Well Binding Plate then centrifuging again. If a higher DNA concentration is required, use 100 µl of pre-heated Elution Buffer then repeat the Elution step by adding the same 100 µl of Elution Buffer (which now contains the eluted DNA) to each well of the Presto™ gDNA 96 Well Binding Plate and centrifuging again.

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.

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.

Vacuum Protocol Procedure

1. Vacuum Manifold Preparation

Set the waste tray in the manifold base. Place the binding top plate on the manifold base. Place a **Presto™ gDNA 96 Well Binding Plate** in the binding top plate aperture. Attach the manifold to a vacuum source.

2. Tissue Dissociation and Lysis

Transfer up to **20 mg of animal tissue (0.5 cm mouse tail) or 1 x 10^6 cultured cells** into each tube of **Microtubes (Racked)**. Add **200 µl of Proteinase K working solution** to each microtube. Seal the tubes with the **Caps for Microtubes (8-strip)** then cover the rack with the plastic cover and mix by inverting. Briefly centrifuge at 3,000 x g to collect any solution from the caps. Allow the centrifuge to reach 3,000 x g prior to stopping. It's important that the tissue samples are completely immersed in Proteinase K working solution. Incubate at 60ºC until the sample lysate becomes clear (at least 6 hours or 10 minutes for cultured cells). Place a weight on top of the plastic cover during incubation. For optimal lysis, mix occasionally or place the rack on a rocking platform during incubation. After incubation, make sure the microtubes are sealed properly. Cover the rack with the plastic cover then mix the sample lysate by shaking vigorously for 10 seconds. Briefly centrifuge at 3,000 x g to collect any solution from the caps. Allow the centrifuge to reach 3,000 x g prior to stopping.

Optional RNA removal step: For RNA-free gDNA, add 5 µl of RNase A (10 mg/ml) into each microtube. Seal the tubes with new caps (not provided). Cover the rack with the plastic cover then mix by shaking vigorously. Briefly centrifuge at 3,000 x g to collect any solution from the caps. Allow the centrifuge to reach 3,000 x g prior to stopping. Incubate for 5 minutes at room temperature.

NOTE: Tissue homogenization prior to incubation will facilitate Proteinase K digestion and cell lysis subsequently increasing DNA yield. Inverting the sample occasionally during incubation will also facilitate Proteinase K digestion and cell lysis. Using an auto shaking system is more convenient when incubating samples. During incubation, pre-heat the required volume of Elution Buffer (400 µl/sample) to 60ºC (for Step 5 DNA Elution).

3. DNA Binding

Carefully open **Microtubes (Racked)** and add **400 µl of Binding Solution** to each microtube. Seal the microtubes with new caps. Cover the rack with the plastic cover then mix by shaking vigorously for 15-30 seconds. Briefly centrifuge at 3,000 x g to collect any lysate from the caps. Allow the centrifuge to reach 3,000 x g prior to stopping. Carefully open microtubes and transfer all of the sample lysate into each well of the **Presto™ gDNA 96 Well Binding Plate** being careful not to get any lysate on the rims of the wells. DO NOT transfer insoluble particles such as hair or bones to the **Presto™ gDNA 96 Well Binding Plate** to prevent membrane clogging. Seal unused wells of the **Presto™ gDNA 96 Well Binding Plate** with adhesive film. Apply vacuum at **15 inches Hg** until samples pass through completely then switch 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 completely (approximately 10 seconds). 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 completely. Apply vacuum for an additional 10 minutes to dry the membrane then switch off the vacuum.

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. Replace the waste tray with Microtubes (Racked). 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] into 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. Seal the microtubes with new caps and store the purified DNA at -20ºC.

1. For the maximum DNA yield, repeat the elution step by adding another 200 µl of pre-heated Elution Buffer to each well of the Presto™ gDNA 96 Well Binding Plate and applying vacuum again. If a higher DNA concentration is required, use 100 µl of pre-heated Elution Buffer then repeat the Elution step by adding the same 100 µl of Elution Buffer (which now contains the eluted DNA) to each well of the Presto™ gDNA 96 Well Binding Plate and applying vacuum again.

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.

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.

Troubleshooting

Low Yield

Improper sample homogenization and lysis

Add 20 µl of Proteinase K to 180 µl of GST Buffer to prepare Proteinase K working solution per sample. Add 200 µl of Proteinase K working solution to each tissue sample, mix well by inverting. Make sure the tissue is completely immerged in the solution. Incubate the sample at 60ºC overnight.

Incomplete buffer preparation.

Add ddH_2O (see bottle label for volume) to dissolve Proteinase K powder and store at 4ºC. Add absolute ethanol (see bottle label for volume) to Wash Buffer prior to use. Be sure and close the bottle tightly after each use to avoid evaporation.

Incorrect DNA elution step.

Use pre-heated Elution Buffer, TE or water (60 ºC) to elute DNA. Ensure Elution Buffer, TE or water is added into the center of the matrix and is completely absorbed. If using water for elution, ensure the water pH is between 7.5 and 8.5. Elute twice to increase the DNA recovery.

Residual RNA Contamination.

Perform the optional RNA removal step.

Clogged column.

Use the recommended amount of starting material. Large amounts of tissue cannot be completely lysed which can clog the membrane and decrease DNA yield and quality.

Undigested tissue has been transferred into wells of binding plate.

DO NOT transfer bones or hairs to binding plates.

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.

The gSYNC™ 96 Well DNA Extraction Kit Test Data

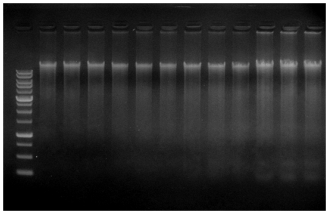


Figure 1. Genomic DNA from 10 mg mouse tissue was extracted using the gSYNC™ 96 Well DNA Extraction Kit. 5 µl aliquots from a 200 µl eluate of purified genomic DNA were analyzed by electrophoresis on a 0.8% agarose gel.
1-3 = kidney
4-6 = muscle
7-9 = liver
10-12 = spleen
M = Geneaid 1 Kb DNA Ladder

	Sample (Mouse)	260/280	260/230	Yield (µg)
1	10 mg Kidney	1.81	2.03	7.4
2		1.82	2.06	8.0
3		1.82	2.07	8.6
4	10 mg Muscle	1.88	1.86	3.0
5		1.89	1.79	4.2
6		1.90	1.73	3.4
7	10 mg Liver	1.81	2.03	7.2
8		1.83	1.98	7.1
9		1.78	1.97	7.3
10	10 mg Spleen	1.83	2.22	13.0
11		1.83	2.16	12.7
12		1.81	2.03	12.0

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

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
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	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

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UKAS QUALITY MANAGEMENT

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