Geneaid

Instruction Manual
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For Research Use Only

Presto™ Plasmid DNA Concentration Kit

PC0002 (2 Preparation Sample Kit)
PC0250/251 (25 Preparation Kit)
PC0500/501 (50 Preparation Kit)
PC1000/1001 (100 Preparation Kit)

Advantages

Sample: Eluate from the Presto™ Midi Plasmid Kit and/or Plasmid Midi Kit
Efficient: replaces traditional, time-consuming DNA precipitation procedures
Binding Capacity: 600 µg of DNA
Recovery: up to 90%
Concentration: up to 3.2 µg/µl
Plasmid Size: 1-20 kb
Elution Volume: 100-400 µl
Dead Volume: 25 µl
Operation Time: 10 minutes/prep (manual), 20 minutes/6 preps (vacuum)
Kit Storage: dry at room temperature (15-25°C) for up to 1 year

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Geneaid Biotech Ltd.
Tel: 886 2 26960999 · Fax: 886 2 26960599 · www.geneaid.com · info@geneaid.com

Introduction

The Presto™ Plasmid DNA Concentration Kit offers a simplified replacement for time-consuming DNA precipitation procedures. The Presto™ Plasmid Concentrator will quickly and efficiently concentrate plasmid DNA in anion-exchange chromatographic DNA purification eluates collected from Geneaid Plasmid Midi Columns, Macherey-Nagel NucleoBond® AX, NucleoBond® Xtra Columns, QIAGEN® tip 500 Columns etc. Plasmid eluates are precipitated with isopropanol then passed through a Presto™ Plasmid Concentrator using a syringe. Once contaminants are removed using a proprietary Desalting Buffer, the concentrator is dried and plasmid DNA is eluted with a small volume (100-400 µl) of low salt Elution Buffer. The Presto™ Plasmid Concentrator eliminates DNA pellet loss and incomplete solubilization of precipitates to ensure highly concentrated, purified plasmid DNA. In only 10 minutes, the plasmid DNA can be used directly in a variety of sensitive downstream applications, such as transfection, sequencing, ligation, PCR, in-vitro transcription, microinjection, restriction enzyme digestion and gene gun.

Quality Control

The quality of the Presto™ Plasmid DNA Concentration Kit is tested on a lot-to-lot basis according to Geneaid's ISO-certified quality management system.

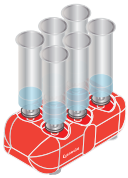
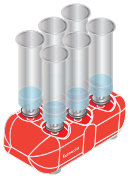
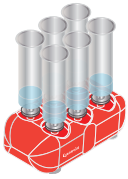
Kit Components

Component	PC0002	PC0250	PC0251	PC0500	PC0501	PC1000	PC1001
Presto™ Plasmid Concentrator	2	25	25	50	50	100	100
Desalting Buffer[1] (Add Ethanol)	0.75 ml (1.75 ml)	9 ml (21 ml)	9 ml (21 ml)	18 ml (42 ml)	18 ml (42 ml)	36 ml (84 ml)	36 ml (84 ml)
Elution Buffer	1 ml	12 ml	12 ml	30 ml	30 ml	60 ml	60 ml
20 ml Syringe	2	25	2	50	2	100	2
1 ml Syringe	2	25	2	50	2	100	2

[1]Add absolute ethanol (see the bottle label for volume) to Desalting 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.

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

Quick Protocol Diagram

	Manual		Vacuum
Precipitate DNA		Add 8 ml of isopropanol to 8 ml of eluate, mix then incubate at RT for 2 mins.	
Load DNA Mixture	 Press slowly	Transfer DNA mixture to a 20 ml syringe attached to a Presto™ Plasmid Concentrator	 Apply low pressure vacuum until mixture passes through completely
Wash		Add 1 ml of Desalting Buffer	
Dry Membrane	 Press fast > 6X		 Turn on vacuum for 5 mins.
Elute DNA	 Press very slowly to elute drop by drop	Elute twice for maximum recovery: 1. 100-400 µl 2. Load first eluate	 Press very slowly to elute drop by drop

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Presto™ Plasmid DNA Concentration 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 Desalting 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.

Additional Requirements

1.5 ml microcentrifuge tubes, isopropanol, absolute ethanol, vacuum manifold

Manual Protocol Procedure

1. Precipitate DNA

Add an **equal volume of room temperature isopropanol to the eluate** from anion-exchange chromatography. For example, add 8 ml of isopropanol to 8 ml of Geneaid Plasmid Midi/ Presto™ Midi Plasmid eluate. Mix well by inverting the tube 8–10 times then incubate at room temperature for 2–5 minutes.

NOTE: If the plasmid sample does not have a high salt concentration (0.2M sodium chloride, 0.3M sodium acetate, or 0.2M potassium chloride), add a 1/10 volume of 3M sodium acetate (pH 4.2) to the sample before adding isopropanol.

2. Load DNA Mixture

Remove the plunger from a 20 ml syringe and attach the **Presto™ DNA Concentrator** to the syringe. Keep the syringe in a vertical position and transfer the DNA mixture to the syringe. Insert the plunger, then **SLOWLY** press the plunger to filter the DNA mixture through the **Presto™ DNA Concentrator** using **minimal but constant force**. Discard the flow-through.

3. Wash

Remove the **Presto™ DNA Concentrator** from the syringe and pull out the plunger. Re-attach the Presto™ DNA Concentrator to the syringe and add **1 ml of Desalting Buffer (make sure absolute ethanol was added)**. Insert the plunger, then wash the membrane by pressing the **Desalting Buffer** through the **Presto™ DNA Concentrator**. Discard the flow-through.

4. Dry Filter Membrane

Remove the **Presto™ DNA Concentrator** from the syringe, pull out the plunger, then re-attach the **Presto™ DNA Concentrator**. Insert the plunger. Dry the **Presto™ DNA Concentrator** by pressing the air through the **Presto™ DNA Concentrator as quickly as possible**. Repeat this step at least 5 more times or until no liquid leaks from the **Presto™ DNA Concentrator**. Dry the tip of the **Presto™ DNA Concentrator** with paper towel.

5. DNA Elution

Pull out the plunger from a 1 ml syringe. Remove the **Presto™ DNA Concentrator** from the 20 ml syringe and attach it to the the 1 ml syringe. Place the tip of the **Presto™ DNA Concentrator** over a 1.5 ml microcentrifuge tube, then add **100-400 µl of Elution Buffer**[1], TE[2] or water[3] into the 1 ml syringe. Insert the plunger, then slowly press the plunger to elute the plasmid DNA drop by drop. Remove the **Presto™ DNA Concentrator** from the 1 ml syringe, pull out the plunger, then re-attach the **Presto™ DNA Concentrator** to the 1 ml syringe. **Transfer the first eluate to the 1 ml syringe, insert the plunger, then elute the plasmid DNA into the same 1.5 ml microcentrifuge again**. Remove the **Presto™ DNA Concentrator** from the 1 ml syringe, pull out the plunger, re-attach the **Presto™ DNA Concentrator**, then press the plunger strongly to force out as much eluate as possible.

NOTE: For maximum recovery, eluting the plasmid DNA twice is strongly recommended.

[1]Elution Buffer (10 mM Tris-HCl, pH8.5)

[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 ≥8.0. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification.

Vacuum Protocol Procedure

1. Precipitate DNA

Add an **equal volume of room temperature isopropanol to the eluate** from anion-exchange chromatography. For example, add 8 ml of isopropanol to 8 ml of Geneaid Plasmid Midi/ Presto™ Midi Plasmid eluate. Mix well by inverting the tube 8-10 times then incubate at room temperature for 2-5 minutes.

NOTE: If the plasmid sample does not have a high salt concentration (0.2M sodium chloride, 0.3M sodium acetate, or 0.2M potassium chloride), add a 1/10 volume of 3M sodium acetate (pH 4.2) to the sample before adding isopropanol.

2. Load DNA Mixture

Remove the plunger from a 20 ml syringe and attach the **Presto™ DNA Concentrator** to the 20 ml syringe. Attach the **Presto™ DNA Concentrator** to a vacuum manifold (such as Vac-Cube™ from Geneaid, QIAvac 24 Plus from Qiagen®, Vac-Man® from Promega). Transfer the DNA mixture to the 20 ml syringe. Apply **vacuum at low pressure** to slowly draw the DNA mixture through the **Presto™ DNA Concentrator**. Turn off the vacuum once the DNA mixture has been completely drawn through.

3. Wash and Dry Filter Membrane

Add **1 ml of Desalting Buffer (make sure absolute ethanol was added)** to the 20 ml syringe. Apply **vacuum at high pressure** to draw the **Desalting Buffer** through the **Presto™ DNA Concentrator**. Continue applying **vacuum for 5 minutes** to dry the filter membrane. Turn off the vacuum, then remove the **Presto™ DNA Concentrator** from the syringe. Dry the tip of the **Presto™ DNA Concentrator** with paper towel.

4. DNA Elution

Pull out the plunger from a 1 ml syringe and attach the **Presto™ DNA Concentrator**. Place the tip of the **Presto™ DNA Concentrator** over a 1.5 ml microcentrifuge tube, then add **100-400 µl of Elution Buffer**[1], TE[2] or water[3] into the 1 ml syringe. Insert the plunger, then slowly press the plunger to elute the plasmid DNA drop by drop. Remove the **Presto™ DNA Concentrator** from the 1 ml syringe, pull out the plunger, then re-attach the **Presto™ DNA Concentrator** to the 1 ml syringe. **Transfer the first eluate to the 1 ml syringe, insert the plunger, then elute the plasmid DNA into the same 1.5 ml microcentrifuge again**. Remove the **Presto™ DNA Concentrator** from the 1 ml syringe, pull out the plunger, re-attach the **Presto™ DNA Concentrator**, then press the plunger strongly to force out as much eluate as possible.

NOTE: For maximum recovery, eluting the plasmid DNA twice is strongly recommended.

[1]Elution Buffer (10 mM Tris-HCl, pH8.5)

[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 ≥8.0. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification.

Troubleshooting

Low Yield

Plasmid sample does not have a high salt concentration.

If the plasmid sample does not have a high salt concentration (0.2M sodium chloride, 0.3M sodium acetate, or 0.2M potassium chloride), add a 1/10 volume of 3M sodium acetate (pH 4.2) to the sample before adding isopropanol.

Elution volume is too small.

Using 100-400 µl of Elution Buffer, TE or water to elute plasmid DNA from the Presto™ DNA Concentrator is recommended. Using less than 100 µl is insufficient to saturate the entire membrane and will reduce DNA recovery.

Elution is too fast.

During elution, minimal force should be used to slowly press the plunger and elute the DNA from the Presto™ DNA Concentrator drop by drop.

Elution step is not performed twice.

For maximum recovery, eluting the plasmid DNA twice is strongly recommended. Fresh Elution Buffer, TE or water should be used to perform the first elution step. The eluate from the first elution should then be used to perform the second elution.

pH of elution buffers is too low.

When using TE buffer or water to elute DNA, make sure the pH is ≥8.0. Using buffer with a lower pH (<7.5) will reduce DNA recovery.

Incorrect preparation of Desalting Buffer.

Add absolute ethanol (see the bottle label for volume) to Desalting 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.

Eluted DNA Does Not Perform Well In Downstream Applications

Residual ethanol contamination.

Manual protocol: after washing the filter membrane with Desalting Buffer, remove the Presto™ DNA Concentrator from the syringe, pull out the plunger, then re-attach the Presto™ DNA Concentrator to the syringe. Insert the plunger. Dry the Presto™ DNA Concentrator by quickly pressing the air through the Presto™ DNA Concentrator. Repeat this step at least 5 more times or until no liquid leaks from the Presto™ DNA Concentrator. Dry the tip of the Presto™ DNA Concentrator with paper towel.

Vacuum protocol: after adding Desalting Buffer to the syringe, continue applying the vacuum at high pressure (15-20 mmHg) to completely remove the ethanol from the filter membrane.

Presto™ Plasmid Concentrator DNA Recovery and Concentration

		Elution Volume			
Input DNA		**100 µl**	**200 µl**	**400 µl**	**600 µl**
100 µg	Recovery	65%	80%	90%	90%
	Concentration	0.9 µg/µl	0.45 µg/µl	0.2 µg/µl	0.15 µg/µl
	Eluate	75 µl	175 µl	375 µl	575 µl
300 µg	Recovery	55%	70%	80%	80%
	Concentration	2.2 µg/µl	1.2 µg/µl	0.65 µg/µl	0.4 µg/µl
	Eluate	75 µl	175 µl	375 µl	575 µl
600 µg	Recovery	40%	60%	75%	75%
	Concentration	3.2 µg/µl	2.1 µg/µl	1.2 µg/µl	0.8 µg/µl
	Eluate	75 µl	175 µl	375 µl	575 µl

Table 1. DNA Recovery is highly dependant on the volume of elution buffer used. 90% recovery can be acheived when using 400 µl of elution buffer. However, DNA concentration is decreased. When using small volumes of elution buffer, recovery is decreased but concentration is increased.

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
Gel/PCR DNA Fragments Extraction Maxi Kit	10/25 preps	DM010/025
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 Genomic DNA Extraction Kit	4/10 x 96 preps	96GB004/010
Presto™ 96 Well Genomic DNA Extraction Kit (Plant)	4/10 x 96 preps	96GP004/010
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
GENEzol™ 96 Well TriRNA Pure Kit	4/10 x 96 preps	96TR004/010
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
Presto™ 96 Well Viral DNA RNA Extraction Kit	4/10 x 96 preps	96VR004/010
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	DL005
100 bp DNA Marker	50 µg, 500 µl	DL006
1 Kb DNA Marker	50 µg, 500 µl	DL007
100 bp + 50 bp DNA Marker	50 µg, 500 µl	DL008
Loading Dye (6X)	10/100 ml	LD010/100
LE Agarose	500 g	AGA500

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

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

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UKAS QUALITY MANAGEMENT

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