ALPHAGEN® BIOTECH CORP.

α+ Solution™ Plasmid Extraction Mini Kit

Kit Contents:

Cat. No:	APPDE 004 (4 preps_sample)	APPDE 050 (50 preps)	APPDE 100 (100 preps)	APPDE 300 (300 preps)
PD1 Buffer	1.0 ml	13 ml	25 ml	75 ml
PD2 Buffer	1.0 ml	13 ml	25 ml	75 ml
PD3 Buffer	1.5 ml	18 ml	35 ml	105 ml
PDW Buffer	1.3 ml	23 ml	45 ml	135 ml
Wash Buffer (concentrate)[a]	1.0 ml	10 ml	20 ml	50 ml
Elution Buffer	0.5 ml	7 ml	15 ml	35 ml
PD Column	4 pcs	50 pcs	100 pcs	300 pcs
Collection Tube	4 pcs	50 pcs	100 pcs	300 pcs
RNase A (Lyophilized)	0.15 mg	1.25 mg	2.5 mg	7.5 mg
User Manual	1	1	1	1
Preparation of Wash Buffer by adding ethanol (96 ~ 100%)				
Ethanol volume for Wash Buffer [a]	4 ml	40 ml	80 ml	200 ml

Specification:

Principle:	mini spin column (silica matrix)
Sample size:	1 ~ 3 ml
Size of plasmid or construct:	< 15 kb
Operation time:	< 25 minutes
Typical Yield:	20 ~ 30 µg
Binding capacity:	60 µg/ column
Column applicability:	centrifugation and vaccum

Important Notes:

1. Store RNase A at -20 °C upon recipit of kit.
2. Add **0.5 ml of PD1 Buffer** to a RNase A tube, Dissolve the RNase A by vortexing. Briefly spin the tube and transfer the total RNase A mixture back to the PD1 bottle, mix well by vortexing and store the **PD1 buffer** at 4 °C.
3. If precipitates have formed in **PD2 Buffer**, warm the buffer in 37°C waterbath to dissolve precipitates.
4. Preparation of **Wash Buffer** by adding 96 ~100% ethanol (not provided) for first use.
5. Centrifugation steps are done by a microcentrifuge capable of the speed at 11,000 ~1,8000 x g.

Brief procedure:

Harvasting

Add **1-5 ml** of bacteria
Centrifuge(~11,000 x g) for 1 min
Discard the supernatant

Cell Lysis

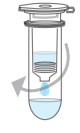
Add **200 µl PD1 Buffer**
Add **200 µl PD2 Buffer**
Add **300 µl PD3 Buffer**

Centrifuge(~18,000 x g) for 5 mins.

Binding

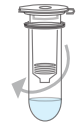
Transfer the supernatant to the PD column
Centrifuge for 30 sec
Discard the flow-through

Wash



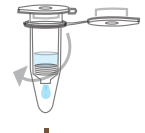
Add **400 µl PDW Buffer**
Centrifuge(~11,000 x g) for 30 sec
Add **700 µl Wash Buffer**
Centrifuge(~11,000 x g) for 30 sec

Dry



Centrifuge(~18,000 x g) for 3 min

Elution



Add **50 – 100 µl of Elution Buffer** or ddH_2O
Stand the column for 2 mins
Centrifuge(~18,000 x g) for 2 mins

Pure plasmid

General Protocol:

Please Read Important Notes Before Starting Following Steps.

STEP	PROCEDURE
1 Harvesting	Transfer **1-3 ml** of well-grown bacteria culture to a microcentrifuge tube (not provided). Descend the bacteria by centrifuging at 11,000 x g for 1 min and discard the supernatant completely.
2 Resuspension	**Add 200 µl PD1 Buffer (RNase A added)** to the pellet and resuspend the cells completely by pipe ng or vortexing. **Note:** • Make sure that RNase A has been added into **PD1 Buffer** whenfirst use. • No cell pellet should be visible after resuspension of the cells.
3 Lysis	**Add 200 µl PD2 Buffer** and mix gently by inverting the tube 5~10 times to lyse the cells and incubate at room temperature for 2 mins until the lysate is homologous. **Note:** **Do not vortex**,vortex may shear genomic DNA. • Do not proceed this step over 5 min.
4 Neutralization	**Add 300 µl PD3 Buffer** and invert the tube 10 times immediately to neutralize the lysate. **Note:** **Do not vortex**, vortex may shear genomic DNA. • Invert immediately after adding **PD3 Buffer** will avoid asymmetric precipitation.
	Centrifuge at full speed(~18,000 x g) for 5 mins. During centrifuging, place a PD Column in a Collecon Tube.
5 DNA Binding	Transfer the supernatant carefully to PD Column. Centrifuge at 11,000 x g for 30 seconds then discard the flow-through. Place the PD column back into the Collection Tube. **Note:** • Do not transfer any white pellet into the column.
6.1 Wash	**Add 400 µl PDW Buffer** to PD Column. Centrifuge at 11,000 x g for 30 seconds then discard the flow-through. Place the PD column back into the Collection Tube.
6.2 Wash	**Add 700 µl Wash Buffer** to PD Column. Centrifuge at 11,000 x g for 30 seconds then discard the flow-through. Place the PD column back into the Collection Tube. **Note:** • Make sure that ethanol (96-100 %) has been added into **Wash Buffer** when first use.
7 Dry column	Centrifuge at full speed (~18,000 x g) for an additional **3 min** to dry the column. **Note:** • Important step ! This step will remove the residual liquid completely that will inhibit subsequent enzymatic reaction.
8 DNA Elution	Place PD Column to a new **1.5 ml** microcentrifuge tube (not provided).
	Add 50 – 100 µl of Elution Buffer or ddH_2O to the membrane center of PD Column. Stand the column for 2 mins. **Note:** For effective elution, make sure that the elution solution is dispensed on the membrane center and is absorbed completely. If plasmid DNA is larger than 10 kb, use preheated 70°C **Elution Buffer** to improve the elution efficiency. Do not Elute the DNA using less than suggested volume (50ul). It will lower the final yield.
9 Pure DNA	Centrifuge at full speed (~18,000 x g) for 2 mins to elute plasmid DNA and store plasmid DNA at 4℃ or -20℃.

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