Seplife® Pd oneS

1. Description

Seplife® Pd oneS resin is designed to specifically bind plasmid DNA (pDNA) and remove RNA, endotoxins, host cell proteins (HCP) and other impurities. In addition, it can effectively separate circular plasmids and supercoiled plasmids and can be used to pre-concentrate supercoiled plasmids. By using pyrogen free water and controlled conditions, it is possible to effectively remove endotoxins and other impurities while purifying pDNA using Seplife® Pd oneS. The resin is easy to operate in gravity column, it can be cleaned and reused maintaining its purification efficiency.

Seplife® Pd oneS is an ion exchange resin made of polystyrene/divinyl benzene (DVB) with large pore size and controlled particle size specifically designed for the purification of plasmid DNA from complex matrixes using gravity columns.

2. Properties

Product	Seplife® Pd oneS
Appearance	White to off-white spherical beads
Type	Ion exchanger
Matrix	Polystyrene / divinyl benzene
Ligand	Quaternary amine
Ion exchange capacity (mmol/g dry)	0.20 - 0.25
Average particle size (μm)	75±10
pH stability	4-12 (operational), 1-14 (cleaning in place)
Chemical stability	Stable in all common buffers; 1 M Sodium hydroxide ; 8 M urea ; 8M guanidine hydrochloride, 75% ethanol, 30% isopropanol. CIP recommended 0.5M NaOH
Loading (mg/ml)*	≥0.25 (high copy plasmid)
Gravity flow rate (cm/h)**	≥60
Recommended operating temperature (ºC)	4-25
Heat resistance	2h in water at 40ºC
Shipped as	20% ethanol slurry

*Test done using high copy plasmid DNA in a 2cm bed height gravity column
**Test done in a 2cm bed height column

3. Instructions

Note: In order to eliminate endotoxins, use endotoxin free (pyrogen free) water throughout the process and operate in controlled conditions. When using prepacked gravity columns, please skip step 3.1.

3.1 Generic column packing instructions

The Seplife® Pd oneS resin is recommended to be packed in a column by gravity. Mix well the resin avoiding using harsh mixing tools or conditions that could damage the particles. Pour the homogenate in the gravity column with the lower distribution plate in place. Fix the upper distribution plate of the column.

3.2 Equilibration

Equilibrate the column by gravity flow with 2-5 times the bed volume (BV) of an equilibration buffer such as: 50 mM Tris-HCl, 0.25 M NaCl, 10 mM EDTA, pH 7.5, so that the conductivity and pH of the effluent are consistent with those of the loading buffer.

3.3 Sample loading

(1) Sample pretreatment: Prepare the sample using alkaline lysis method. It is recommended to resuspend the bacterial sludge at a ratio of 5:1 (volume to mass ratio), then add 0.2M NaOH and 1% SDS for 4-10 min under slow stirring. During the alkaline lysis process, it is important to control the addition of the alkaline solution and the contact time to avoid irreversible damage to the plasmid and affect the recovery rate of the pDNA. Finally, use 3M potassium acetate pH 5.5 to neutralize the lysate. After standing for 30 minutes , centrifuge the lysate to obtain the supernatant or use membrane filtration to clarify before loading on the column.

(2) Sample loading: Samples are usually prepared and used immediately. It is not recommended to use samples that have been stored for a long time, as this will affect the purification effect.

3.4 Washing

After sample loading, use a wash buffer to remove RNA, endotoxins, HCP and other impurities. To maximize sample purity, it is recommended to use 3-5 BV or higher . Example of wash buffer: 50mM Tris-HCl, 0.45 M NaCl, 10mM EDTA, pH 7.5.

3.5 Elution

(1) Example of elution buffer: 50mM Tris-HCl, 1.25M NaCl, 10mM EDTA, 15% isopropanol, pH 8.5. It is recommended to use 1-2 BV of elution buffer to ensure an efficient recovery of plasmid DNA.

(2) Eluted sample processing: Add isopropanol to the elution fraction to a final concentration greater than

30% to encourage pDNA precipitation. After standing at 4ºC for 30 min, centrifuge at ⩾10,000 G for 20 min, and then gently remove the supernatant (no shaking to reduce loss). Quickly add 1/4 of the elution volume of 70% or anhydrous ethanol to wash the pDNA sample. After standing at 4ºC for 30 min, centrifuge at ⩾ 10,000 G for 30 min. Finally, gently remove the supernatant (no shaking to avoid removing solid particles). Completely evaporate the ethanol in the centrifuge tube and add ultrapure water or nucleic acid preservation buffer to re-dissolve the precipitated pDNA and store for later use.

3.6 Cleaning in Place (CIP)

After pDNA elution, rinse the gravity column with 3 BV ultrapure water, then use 0.5M NaOH solution to perform CIP in situ by soak it in 2 BV 0.5M NaOH solution for 15-30 minutes, or use 5 BV 0.5M NaOH solution for dynamic cleaning. After the CIP is completed, rinse the resin with ultrapure water to remove all alkaline solution.

4. Storage

Store in closed containers at 4-30℃, in a dry, ventilated and clean place, away from direct sunlight. Do not freeze. Used gravity columns should be stored at 4-30℃ in 20% ethanol solution.

5. Transportation

Avoid sunlight, rain, and heavy pressure during transportation. It is strictly forbidden to transport with toxic and hazardous materials.

6. Precautions

(1) All buffers should be sterilized or sterile filtered before use to avoid contamination.

(2) After the gravity column is filled, please strictly follow the cleaning process before storage to maintain the service life of the resin.

(3) It is recommended to select a gravity column tube of appropriate size according to different needs. Avoid drying the resin and using mixing tools that could damage the beads. It is recommended to shake well the slurry before loading the column.

(4) The sample concentration should not be too high. High concentration under gravity conditions can easily cause sample loss.

(5) If there are strict requirements for endotoxin, please use pyrogen-free water to prepare all buffers, sterilize all equipment and conduct experiments in a clean room, and follow the transfection-grade plasmid

preparation process in the operating procedures and instructions .

7. Ordering information

Product Name	References	Pack Size
Seplife ® Pd oneS	PS33135M2-2	25ml
	PS33135M2-3	100ml
	PS33135M2-4	500ml
	PS33135M2-5	1L
	PS33135M2-6	5L
	PS33135M2-7	10L

Product Name	Gravity Columns Name	References	Columns Size	Resin Bed Volume
Seplife ® Pd oneS Gravity Columns	PGRapid Pd oneS S	PG33135M2-2	30mL	6ml
	PGRapid Pd oneS M	PG33135M2-3	60mL	12ml
	PGRapid Pd oneS L	PG33135M2-4	150mL	40ml
	PGRapid Pd oneS XL	PG33135M2-5	300mL	80ml

Production date: See label

Service life: 2 years, under proper storage conditions

Picture of the gravity columns. From left to right:
PGRapid Pd oneS S; PGRapid Pd oneS M; PGRapid Pd oneS L; PGRapid Pd oneS XL.

Manufacturer: Sunresin New Materials Co. Ltd.
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