
NMacro dT25 & NMacro dT25-S

Oligo dT Affinity Chromatography Resins

Product Instruction

NMacro dT25 & NMacro dT25-S

Oligo dT affinity chromatography resins

NMacro dT25 and NMacro dT25-S are two affinity resins specifically designed for the high efficiency capture of large mRNA molecules through base pairing with the mRNA polyA tail. They feature rigid PS-DVB bead matrices of large through pore structure for efficient mass transfer. Their matrices have hydrophilic poly-hydroxyl surface, resulting in low non-specific binding. Their surface is coupled with polyT (oligo dT25) ligand with an alkali-resistant chemical bond, enabling the resin to have excellent stability in high pH CIP conditions and long lifetime. The resins are also stable at elevated temperatures for the breakdown of undesired higher order structures, if required. NMacro dT25 has very large pore morphology and exhibits superior binding capacity for very large mRNA molecules (>5000nt), while NMacro dT25-S has relatively smaller pore size providing better binding capacity for small to medium size mRNA (<5000nt).

Highlights of NMacro dT25 and NMacro dT25-S resins are:

- Rigid matrix of large through-pores for efficient mass transfer enables to operate as fast flow for high productivity.
- Withstand harsh conditions such as 0.5M NaOH and high temperature, ensuring consistent performance in various environments and long lifetime.
- Two resins of different pore sizes complementarily provide excellent binding capacities for small, medium to very large size mRNA respectively.

Table 1. Technical parameters for NMacro dT25

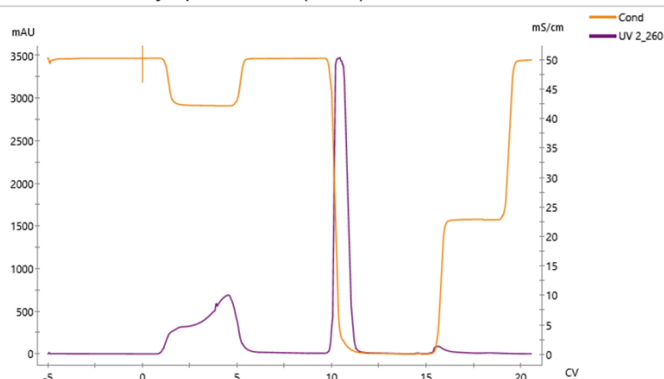
Product name	NMacro dT25	NMacro dT25-S
Matrix	PS/DVB beads	
Particle size	50 μ m	
Pore size	Very large	Relatively small
Ligand	dT-25mer	
Dynamic binding capacity of PolyA 300mer	~1 mg/mL	~4.5mg/mL
Maximum pressure	3 MPa	
CIP	0.1- 0.5M NaOH	
Recommended flow rate	50-300 cm/h	
pH stability	2-13	
Chemical stability	Stable in various chromatography solutions, such as pure water, acetonitrile, 2 M acetic acid, 1 M HCl, 0.5M NaOH, ethanol, and other common organic solvents. Avoid exposure to strong oxidizers, oxidizing acids.	
Storage	20% ethanol, 2-8 $^{\circ}$ C	

Chromatography application

NMacro dT25 and NMacro dT25-S were developed to meet the needs of large-scale downstream purification of large mRNA molecules used in vaccine and gene therapy with good selectivity and high binding capacity. These resins capture mRNA through a simple process involving the binding of polyadenylation (poly A) tails in a saline solution and elution by pure water. Figure 1 provides an example of capturing mRNA (1000 nt) using NMacro dT25 and NMacro dT25-S

respectively. The mRNA with a poly A tail binds to the resins in a buffer with 0.5 M NaCl, and elutes from the resin due to the repulsion of negative charges between the mRNA and the resin ligands when the mobile phase contains no salts.

NMacro dT25 affinity capture of mRNA (1000nt)



NMacro dT25-s affinity capture of mRNA (1000nt)

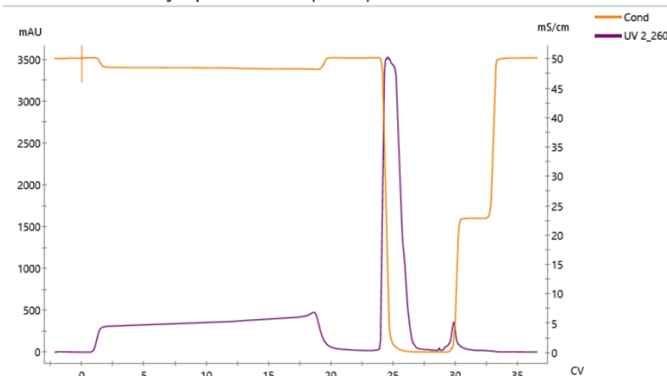


Figure 1. Chromatogram of capturing of mRNA (1000 nt) using NMacro dT25(top) and NMacro dT25-S (bottom) respectively.

Column packing

Determinate the concentration of slurry

NMacro dT25 and NMacro dT25-S are stored in its original packaging containing 20% ethanol. The concentration of slurry (Cs) is about 65% (v/v) (1.5 Kg of the product homogenate corresponds to 1 L of resin volume). If the concentration of slurry is unknown, it can be determined by pouring 10 mL shaken resin into a measuring cylinder for overnight (typically 12 to 24 hours) and calculating the ratio of gravity settling volume (Vr, unit: mL) to origin volume (10 mL).

$$Cs (\%) = Vr/10 \times 100\%$$

To achieve the best column packing result, it is recommended to prepare a 50~70% resin slurry using a 0.5 M NaCl solution.

Calculate the required volume of resin slurry

Calculate the column volume (Vc) by the following formula:

$$Vc = \pi r^2 \times h$$

Where:

h, column height.

r, radius of the column.

Calculate the required volume of resin slurry the following formula:

$$Vs = 100 \times (Vc \times CF) / Cs$$

Where:

Vs, required volume of resin slurry.

CF, Compression factor.

Recommended resin compression factor: 1.05-1.10.

Prepare resin slurry

Lab-scale small column (I.D. ≤ 50 mm):

Subject the calculated amount of resin slurry in 20 % ethanol to suction filtration, add the loading solution (in 3 times the volume of the slurry) into the filter funnel, gently stir with a plastic spoon and then perform suction filtration again. Repeat the above-mentioned procedure twice so that the 20% ethanol in the medium slurry is completely replaced by the loading solution. Finally, add loading solution to make up the total volume of the slurry to 1.67~2 times the target column volume (Cs: 50%-60%).

Larger scale column (I.D. > 50 mm):

Measure and transfer the calculated amount of resin slurry in 20 % ethanol into a homogenate tank and allow the slurry to settle for more than 2 hours, then remove the supernatant (which might be a bit turbid). Replenish loading solution in the same volume of the removed supernatant. Gently stir the slurry and then allow it to settle for more than 2 hours, then remove the supernatant. Repeat the above-mentioned procedure twice or thrice so that the ethanol is completely replaced by the loading solution. Finally, add loading solution to make up the total volume of the homogenate to 1.67~2 times the target column volume (Cs: 50%-60%).

Note: During the preparation of the homogenate for column-packing, avoid grinding or rubbing the resin; do not use magnetic stirrer or silicone tube peristaltic pump; if mechanical agitator is used, the paddle should not be too close to the column wall. Furthermore, when it is necessary to re-pack the column, homogenate should be prepared according to the above-mentioned filtration or settlement procedure.

Packing steps

Flow packing of column:

- Thoroughly homogenize the above-mentioned medium suspension and transfer it into the column tube.
- Start the column-packing with the loading solution as mobile phase (the effluent may be a bit turbid at the beginning of the column-packing but will become clear after the flow of 1-2 CV of loading solution).
- Note: The starting flow rate for column packing should not be too high.
- When the column bed height stabilizes, increase the flow rate to maximum target value (Recommended value: 2 times the operation flow rate) or increase the column-packing pressure to maximum target value (Recommended value: NMT 1.0 MPa). Once the pressure becomes constant, allow the column-packing to stabilize for another 20 minutes.
- Simply adjust the piston to 2-3mm below the gel surface.

Axial compression packing of column (I.D.>300 mm):

- Clean and degas the column.
- Connect the homogenate tank to the column, remove the surplus solution in the column, lower the piston to 5cm away from the lower frit.
- Open the feeding valve, lift the piston for draw-off. Draw-off rate: 200~300 cm/h. Determine the draw-off amount according to the homogenate concentration, and calculate the piston travel distance. Close the feeding valve, move the piston downwards for column compression at a compression rate of 60~100 cm/h.
- Keep compressing until the complete settlement of filler; stop the piston when the column bed height no longer increases, then record the column bed height. Calculate the final position of the piston with a compression ratio of 1.05.

Note: Generally, the mass of resin is calculated using a percentage over 120% since the ingress of air during the slurry draw-off is inevitable.

Evaluate column efficiency

After the column is packed, equilibrate the column with 2 CV 0.5 M NaCl solution and conduct column efficiency test at a flow rate of 100 cm/h. For the specific test parameters, refer to Table 2:

Table 2. Conditions for column efficiency evaluation.

Sample	2 M NaCl solution
Loading volume	1.0-5.0% CV
Mobile phase	0.4 M NaCl solution
Flow rate	50-200 cm/h
Detection	Conductivity
Recommended qualification	As: 0.8-1.5
specifications	Plates (N/m) : >2500

Chromatography method

Recommended loading condition

pH: Both T-A base pairing binding and elution occur under neutral pH conditions.

Buffer: Typically, phosphate and Tris-HCl buffer systems are utilized. In selecting a buffer system, it is crucial to consider molecular stability, optimization of binding, and the buffer's capability to maintain the pH within the desired operational range.

Conductivity: Generally, a concentration of at least 250-500 mM NaCl is employed, as high conductivity aids in enhancing the base pair binding capabilities.

Flow rate: While the target operating flow rate is adjustable, to attain optimal binding condition, a retention time of over 5 minutes is recommended (assuming a column with a height of 20 cm, implying a linear flow rate of less than 400 cm/h).

Temperature: Affinity binding occurs at room temperature. However, if necessary, the total RNA loading samples can be heated to 65-70°C to disrupt the secondary structure.

Equilibration: It is recommended to use 5 CV or more equilibrium buffer to equilibrate the column.

Binding condition optimization: The maximum binding capacity depends on several factors, including molecule size, sample

concentration, impurities, and conductivity.

Recommended elution condition

pH: Typically, the initial pH of the elution step aligns with the loading buffer and equilibrium buffer. However, if required, the pH of the eluent can be adjusted for optimization purposes.

Note: The OD value of the mRNA eluent, which is utilized for determining concentration, will vary depending on the different pH levels.

Elution conductivity: In the absence of salts (low conductivity) in the elution buffer, electrostatic repulsion occurs between the poly A and poly T anionic backbones, leading to the dissociation of A-T base pairs and the subsequent elution of poly A-mRNA. In some cases, water alone can be used for elution; typically, a salt concentration gradient is not required. However, reducing the conductivity during intermediate wash steps can enhance the removal of impurities.

Yield: The yield of mRNA during elution depends on the optimization of specific wash conditions and the molecular characteristics.

CIP: Typically, 3-5 CV of pure water or 20% ethanol is used for CIP. If necessary, 3-5 CV 0.1 M NaOH can be used for CIP, followed by rinsing with pure water or 20% ethanol to remove the alkaline solution.

Recommended storage condition

The resin is recommended to store in in a sealed condition in 20% ethanol at 2-8 °C. To prevent ethanol evaporation and microbial growth, it is recommended to replace it with fresh 20% ethanol every 3 months.

Order Information

Product	Package	Cat.NO
NMacro dT25	10 mL	17031-050500-2010
	30 mL	17031-050500-2030
	50 mL	17031-050500-2050
	100 mL	17031-050500-2100

	300 mL	17031-050500-2300
	500 mL	17031-050500-2500
	1 L	17031-050500-1001
NMacro dT25-S	10 mL	17031-050500-2010
	30 mL	17031-050150-2030
	50 mL	17031-050150-2050
	100 mL	17031-050150-2100
	300 mL	17031-050150-2300
	500 mL	17031-050150-2500
	1 L	17031-050150-1001

We also offer 7.7 mm × 22 mm, 16 mm × 25 mm, 7.7 mm × 100 mm preppacked columns. More requirements about products or customized needs, please contact us.

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