
NanoGel dT20

Oligo affinity Chromatography Resin

Product Instruction

NanoGel dT20

Oligo affinity chromatography resin

NanoGel dT20 affinity resin is specifically designed for the purification of mRNA. It features a rigid PS-DVB bead matrix, which offers uniform particle size and a large open pore structure. This bead matrix is coated with a poly-hydroxyl surface, resulting in low non-specific binding. The surface is further functionalized with poly oligo (dT20), enabling the resin to capture mRNA through base pairing with the poly A tail of mRNA.

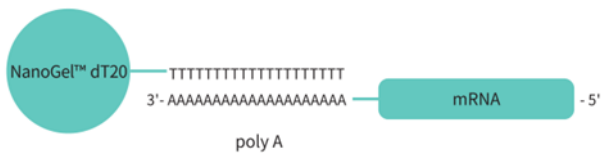


Figure 1. Schematic diagram of NanoGel dT20 structure and its binding with mRNA

We produce NanoGel dT20 with high-quality raw material through a proprietary process with stable microstructure, enabling precise control over particle size, pore size, and porosity. The resin has the following characteristics:

- Rigid matrix enables to operate as fast flow for high productivity.
- Highly competitive dynamic binding capacity.
- Mono-sized matrix yields the robust performance in varied applications including not only bioprocessing.
- Withstand harsh conditions such as high salt, high pH, and high temperature, ensuring consistent performance in various environments.
- Higher manufacturing productivity meets the purification needs of different biological products.

Table 1. Technical parameters for NanoGel dT20 resin.

Product name	NanoGel dT20
Matrix	PS/DVB beads
Particle size	50 μm
Ligand	dT-20mer
Dynamic binding capacity	~2 mg/ml (mRNA)
Maximum pressure	3 MPa
CIP	0.1 M 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, ethanol, and other common organic solvents. Avoid exposure to strong oxidizers, oxidizing acids, strong reducing agents, acetone, benzyl alcohol, or tetrahydrofuran (THF).
Storage	20% ethanol, 2-8 °C

Chromatography application

NanoGel dT20 resin is developed to meet the needs of large-scale downstream purification of a wide range of mRNA used in vaccine and gene therapy with good selectivity and high binding capacity. The resin captures mRNA through a simple process involving the binding of polyadenylation (poly A) tails in a saline solution and elution by pure water. Figure 2 provides an example of capturing mRNA (1000 nt) using NanoGel dT20. The mRNA with a poly A tail binds to the NanoGel dT20 resin 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. The yield of mRNA is above 70%.

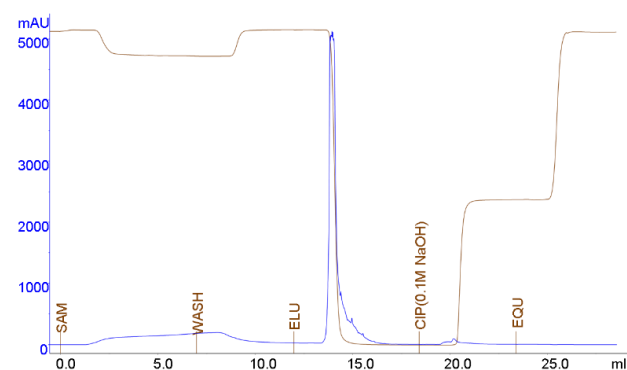


Figure 2. Chromatogram of capturing of mRNA (1000 nt) using NanoGel dT20

Improved alkaline stability allows better process economy

To assess the alkaline stability of NanoGel dT20, the dynamic binding capacity of the resin was tested every 10 cycles by undergoing CIP with 0.1 M NaOH for 30 minutes in each cycle. After 100 such cycles, the dynamic binding capacity had decreased by only 15.6%. It indicates that the resin possesses excellent alkali stability, resulting in better process economy.

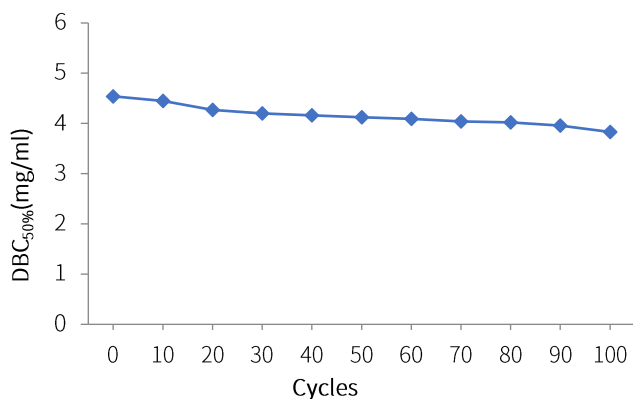


Figure 3. Alkaline stability study of NanoGel dT20 (lot L11W281A) by using 0.1 M NaOH at room temperature

Column packing

Determinate the concentration of slurry

NanoGel dT20 is 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 specifications	As: 0.8-1.5 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.

Storage

The resin is recommended to store 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 name	Package	Cat. No.
NanoGel dT20	10 mL	17030-050150-2010
	30 mL	17030-050150-2030
	50 mL	17030-050150-2050
	100 mL	17030-050150-2100
	300 mL	17030-050150-2300
	500 mL	17030-050150-2500
	1 L	17030-050150-1001

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

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