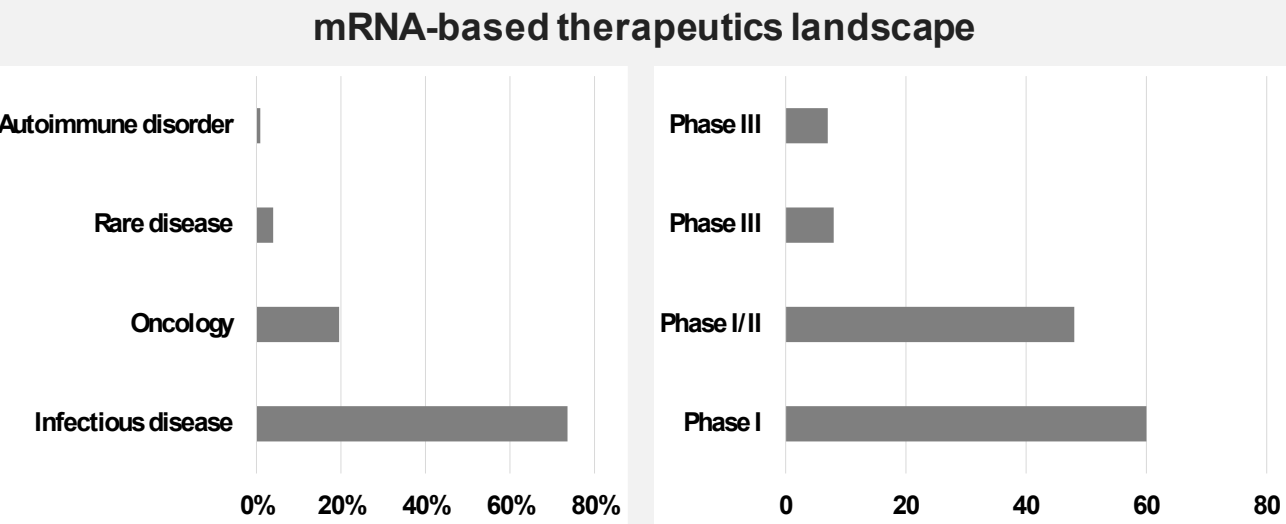


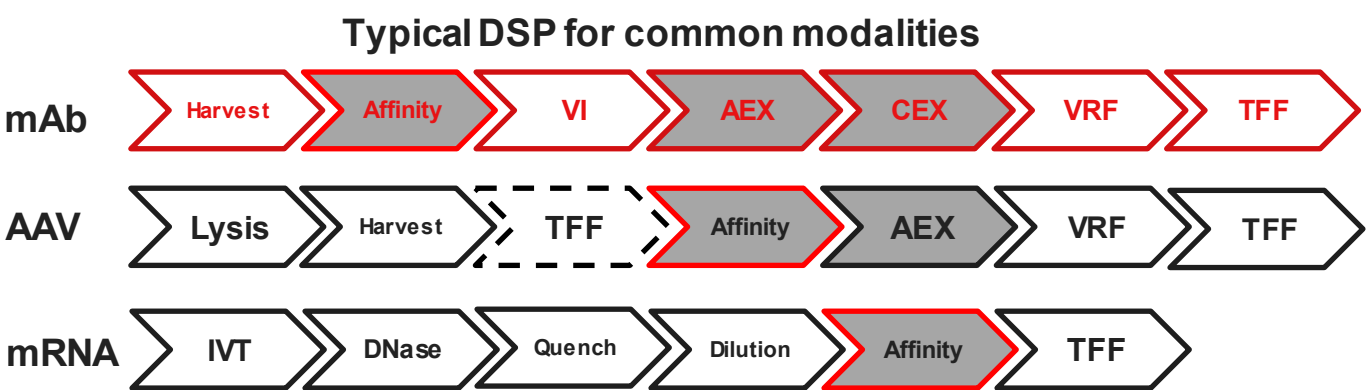
Process and facility-fit calculations for Oligo dT affinity chromatography in mRNA manufacturing

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Introduction



- 4 FDA approved mRNA vaccines and ~300 ongoing clinical trials globally¹
- ~70% of mRNA applications focused on Infectious diseases²



- Simpler downstream process (DSP) for mRNA compared to other modalities
- Single chromatography step using oligo dT (ODT) affinity

Goals

- Assess process development and scale up considerations for ODT products: Thermo Scientific™ POROS™ Oligo (dT)25 Affinity Resin and competitor
- Process calculations for industry-relevant GMP manufacturing case scenarios

Methodology

Dynamic binding capacity: POROS Oligo (dT)25 data were generated internally for constructs with 2500 nt and 3000 nt length. Additional POROS and competitor data as function of residence time (RT) were compiled from references 3 - 5.

Pressure-flow curves: POROS Oligo (dT) 25 were generated using a 7 cm i.d. column packed to 20 cm bed height and using 0.1 M NaCl as mobile phase. Additional curves were calculated using Kozeny-Carman equation⁶.

Operational flow rates: GMP pre-packed column sizes and pressure/flow rate limitations were based on literature from multiple vendors.

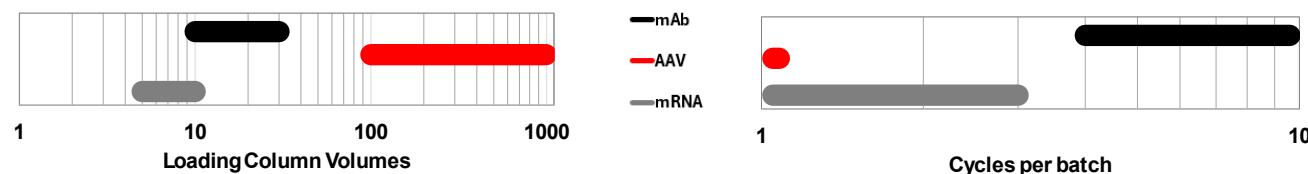
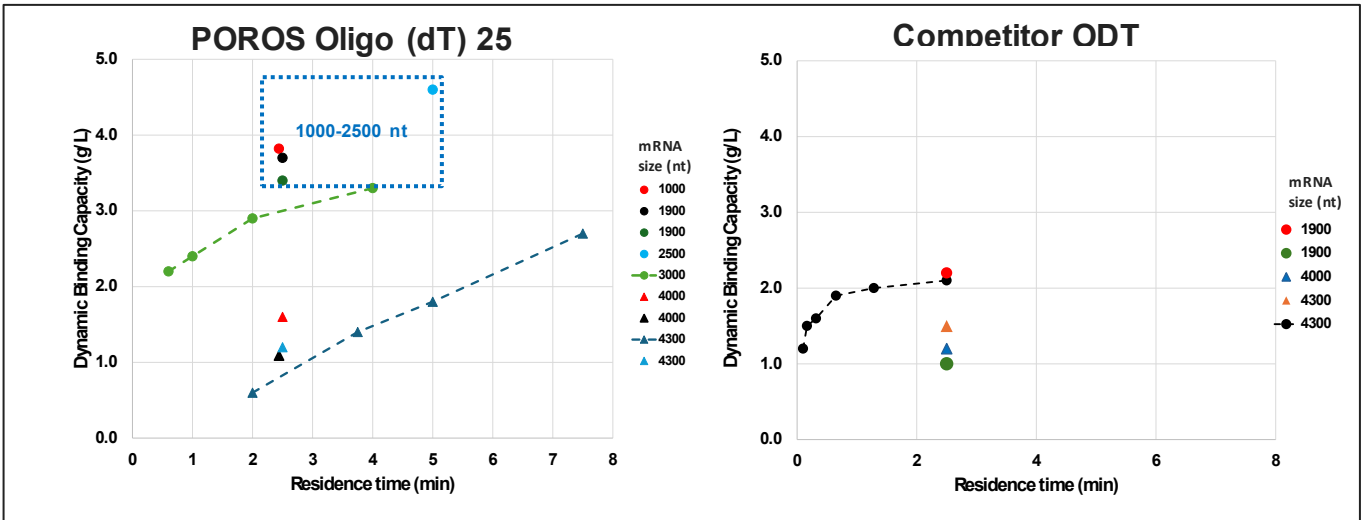
Case scenarios (hypothetical): Microsoft™ Excel™ was used to perform the process calculations and the results were further analyzed and plotted using JMP™ software.

References

- Moschioni, et al. Human Vaccines & Immunotherapeutics, (2026);
- Li, et al. Human Vaccines & Immunotherapeutics, (2026);
- Flook, K. Cell & Gene Therapy Insights (2021);
- Dewar, et al. J Chrom A (2024);
- Tan, et al. Biochem. Eng. J. (2024);
- Carta and Jungbauer, Protein Chromatography: Process Development and Scale Up, 2nd Ed. (2020).

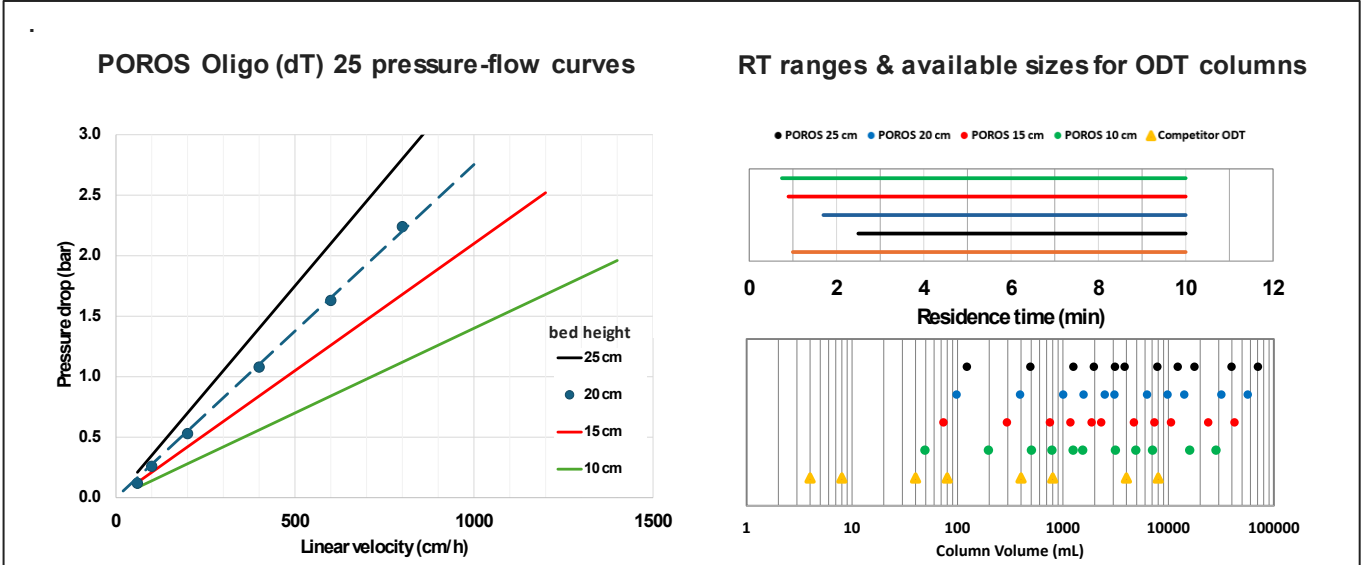
Results

Dynamic binding capacity (DBC), loading column volumes (CV) and cycles per batch



- POROS Oligo (dT)25: DBC > 3.0 g/L for mRNA constructs ≤ 2500 nt at 2.5 min RT
DBC >2.5 g/L for 3000 - 4300 nt constructs at 4-6 min RT
- Competitor ODT: DBC ≤ ~2 g/L irrespective of mRNA construct size and RT
- Lower loading CVs and less cycles per batch in mRNA affinity step allow for longer load step RT and increased DBC without significant impact to process time

Operational flow rates and column sizes



- Linear velocities > 700 cm/h possible for POROS Oligo dT(25) with bed heights ≤ 25 cm
- 1-2 min RT for non-loading steps possible for POROS Oligo dT(25) and competitor
- Larger number of GMP column size options for POROS Oligo dT(25)
- CV sizes >8 L available for POROS to accommodate for higher mRNA demands

Case scenario assumptions

- POROS Oligo (dT)25 dimensions: 8-60 cm I.D., 10, 15, 20, 25 cm bed heights
- Competitor ODT: 4 L and 8 L discrete sizes

Operation parameters

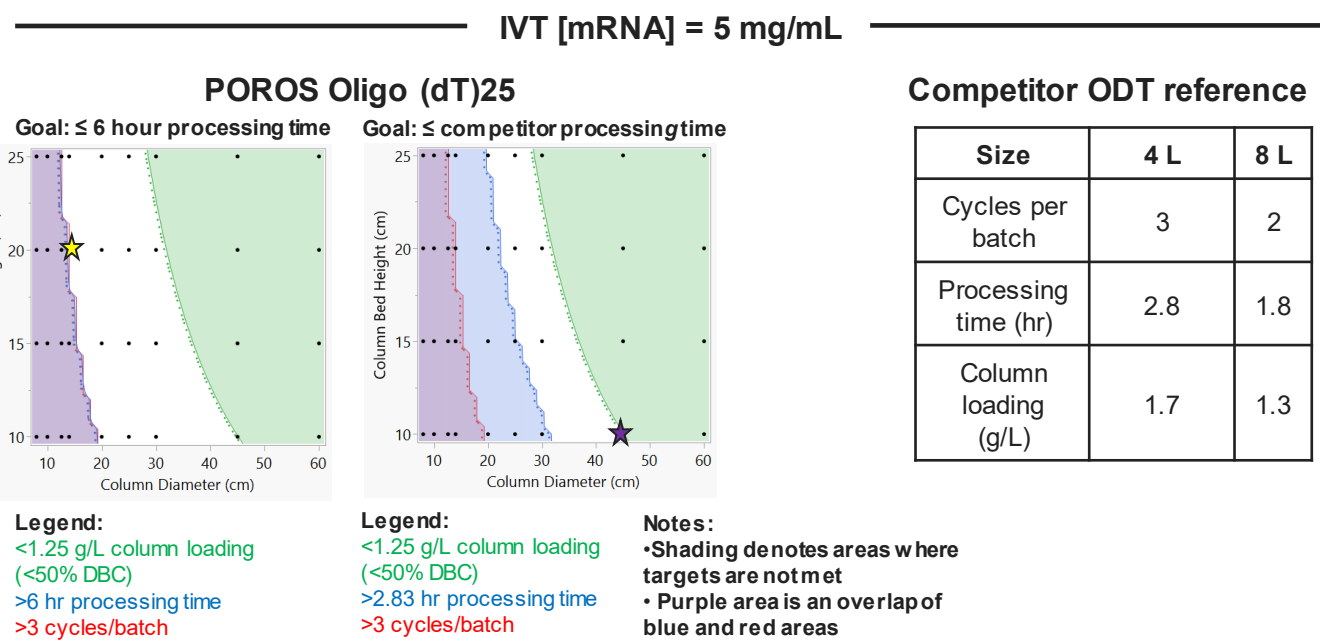
Step	Competitor ODT		POROS Oligo (dT)25	
	CV	RT (min)	CV	RT (min)
Equilibration	10	1	5	Variable, Based on Bed Height
Loading	≤2 g/L	1	≤2.5 g/L	6
Wash	10	1	5	Variable, Based on Bed Height
Elution	10	1	5	
Clean	10	1	5	
Storage or Re-EQ	10	1	5	

POROS Oligo (dT) 25
Non-loading steps flow rates

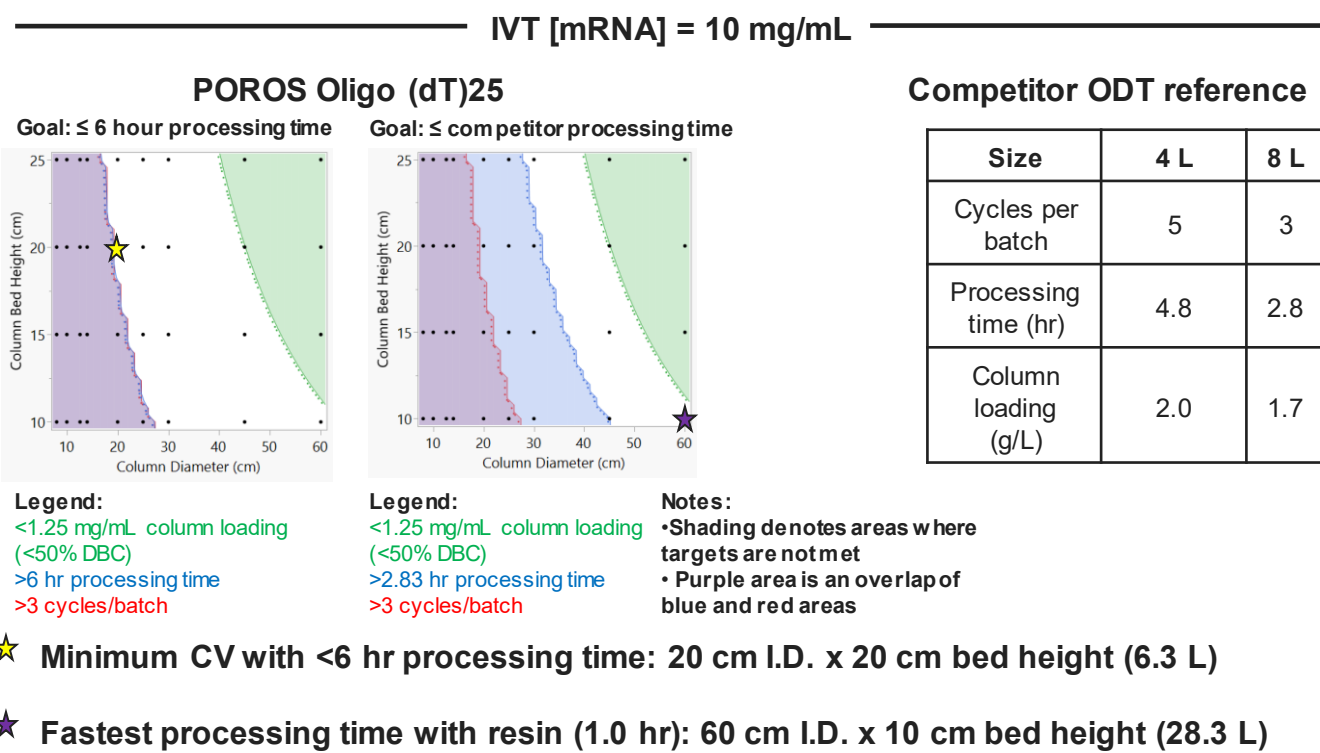
Bed Height (cm)	Linear Velocity (cm/h)	RT (min)
10	600	1
15		1.5
20		2
25		2.5

Clinical manufacturing process case scenario

- IVT reaction: 4 L at 5 OR 10 mg/mL mRNA. Diluted to 0.25 mg/mL for affinity purification
- Process goals: ≤3 cycles, ≥50% DBC loading, ≤6 hours processing time



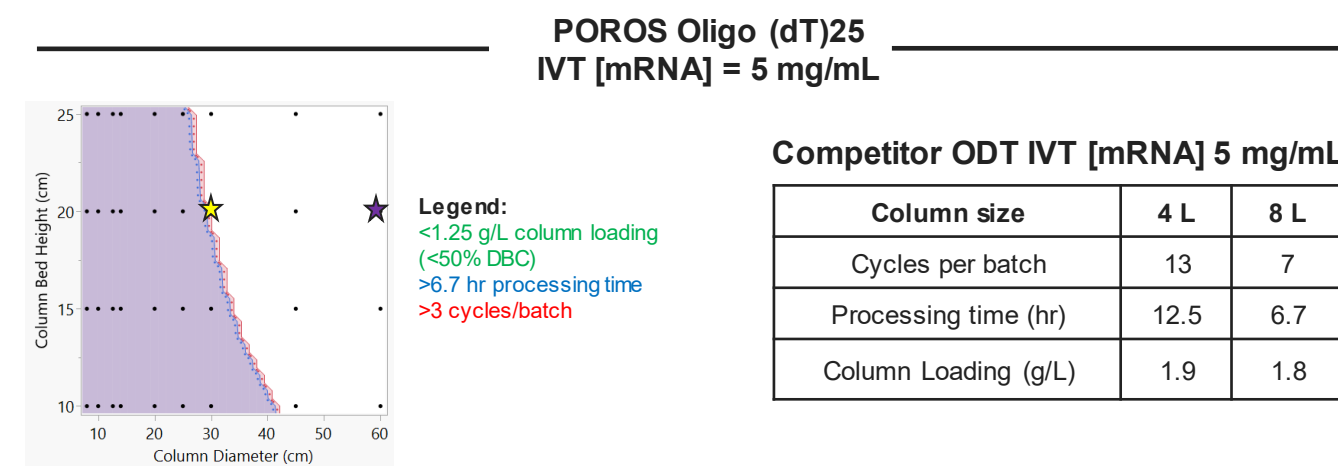
- Minimum CV with <6 hr processing time: 14 cm I.D. x 20 cm bed height (3.1 L)
- Fastest processing time with resin (1.3 hrs): 45 cm I.D. x 10 cm bed height (15.9 L)



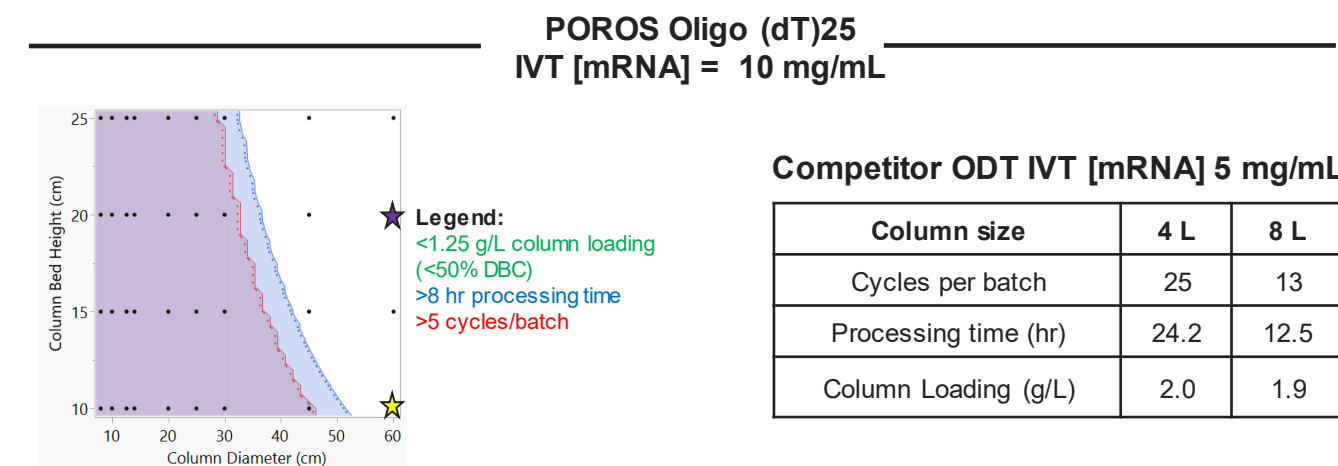
- Minimum CV with <6 hr processing time: 20 cm I.D. x 20 cm bed height (6.3 L)
- Fastest processing time with resin (1.0 hr): 60 cm I.D. x 10 cm bed height (28.3 L)

Commercial manufacturing process case scenario

- IVT reaction: 20 L at 5 or 10 mg/mL mRNA. Diluted to 0.25 mg/mL for affinity purification
- Process goals (5 mg/mL [mRNA]): ≥50% DBC loading, ≤3 cycles, ≤competitor ODT processing time
- Process goals (10 mg/mL mRNA): ≥50% DBC loading, ≤5 cycles ≤8 hours processing time



- Minimum POROS CV with <6.7 hrs processing time: 30 cm I.D. x 20 cm bed height (14.1 L)
- Fastest processing time with POROS resin (1.5 hrs): 60 cm I.D. x 20 cm bed height (56.5 L)



- Minimum POROS CV with <8 hrs processing time: 60 cm I.D. x 10 cm bed height (28.3 L)
- Fastest processing time with POROS resin (3.1 hrs): 60 cm I.D. x 20 cm bed height (56.5 L)

➔ At IVT [mRNA] 10 mg/mL processing time exceeds 12 hrs with largest competitor ODT

Conclusions

- POROS Oligo (dT) 25 achieves binding capacities >2.5 g mRNA/L resin at RT >5 min without impacting overall processing time in the affinity capture step.
- A broad range of column sizes and operating flow rates for non-loading steps further allow optimization of processing time, resin utilization, and/or cycles per batch.
- In both example case scenarios, comparable processing times to competitor ODT can be achieved using flexible column dimensions. Further process goals for cycle number, resin utilization and reduced processing time can also be achieved.

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POROS Oligo dT(25) Affinity Resin: "For research and development use and further manufacturing in support of FDA-regulated end uses. Not for diagnostic use or direct administration to humans or animals."