

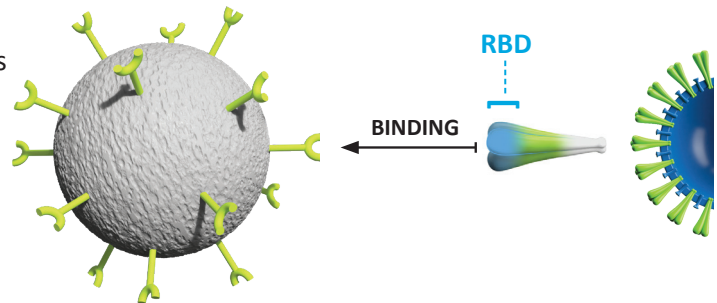
# Streamline purification of SARS-CoV-2 Spike Protein RBD

The NGL COVID-19 Spike Protein Affinity Resin binds the SARS-CoV-2 Spike Protein receptor binding domain (RBD) to yield exceptional purity in a single chromatography step. The resin meets all expected release testing for GMP manufacturing requirements, including bioburden, and provides high selectivity, high dynamic binding capacity, and caustic stability over multiple cycles.

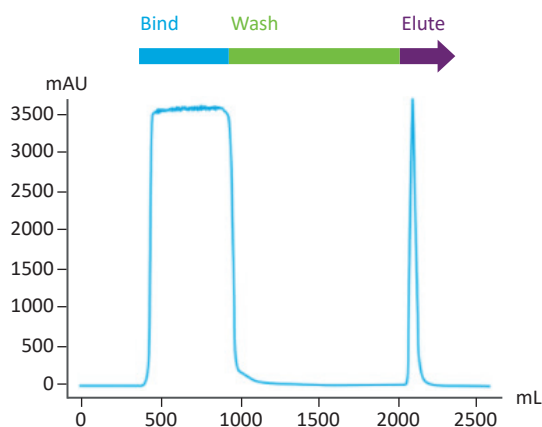


The NGL COVID-19 Spike Protein Affinity Resin utilizes a ligand developed in partnership with Navigo Proteins, a leader in custom affinity chromatography solutions. Repligen manufactures the affinity ligand, couples the ligand to the solid support, performs quality control, and supports application and commercial development. Off-the shelf, pre-packed and pre-qualified OPUS® Columns enable rapid implementation; loose resin affords a self-pack option.

- Targets RBD of (S1/S2), S1 Protein, trimer modalities
- > 90% purity in a single chromatography step
- > 15 mg/ml dynamic binding capacity (DBC)
- Sustains > 88% DBC over 50 CIP cycles



## Simplify RBD Purification with Affinity Chromatography: Bind, Wash, Elute



	OPUS® RoboColumn®	OPUS® MiniChrom®	OPUS® ValiChrom®	OPUS® 2.5 - 80R
Volume	50 µL - 600 µL	0.2 mL - 10 mL	2 mL - 300 mL	25 mL - 150 L
Column capacity*	750 µg - 9 mg	3 mg - 150 mg	30 mg - 4.5 g	375 mg - 2,250 g

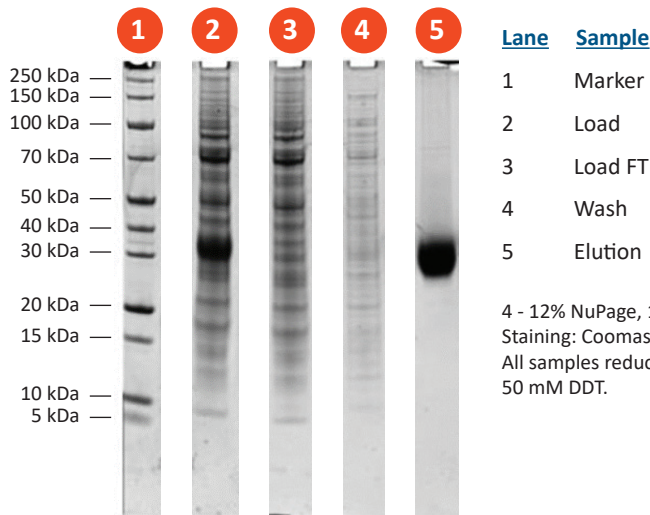
\* Column capacity based on a DBC of 15 mg/ml.

RBD purified from CHO cell culture using NGL COVID-19 Spike Protein Affinity Resin packed in an OPUS column. Column equilibration (5 CV PBS, 0.5 CV) was followed by bind (17 g/L, 4 min rt), wash (5 CV PBS; 5 CV PBS + 1 M NaCl; 5 CV PBS at 0.5 CV/min), and elution (0.1 M acetic acid, pH 3.5 at 0.5 CV/min). The broad portfolio of OPUS® Columns enables rapid scale-up from process development to GMP manufacturing of purified RBD.



# Achieve high purity protein that binds ACE-2

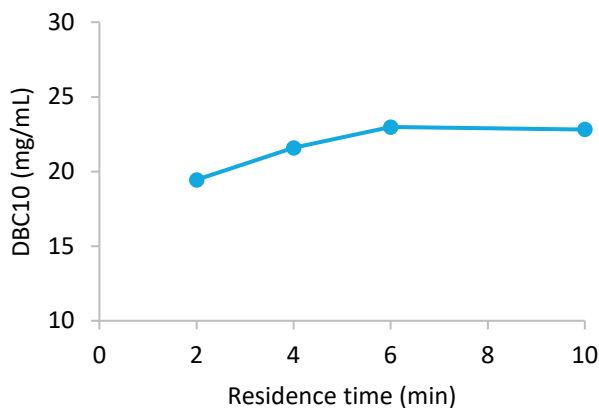
The NGL COVID-19 Spike Protein Affinity Resin efficiently captures SARS-CoV-2 receptor binding domain (RBD) containing variants from cell culture media and purifies to > 90% purity in one chromatography step. Mild elution conditions yield functional protein that retains high affinity to the ACE-2 target.



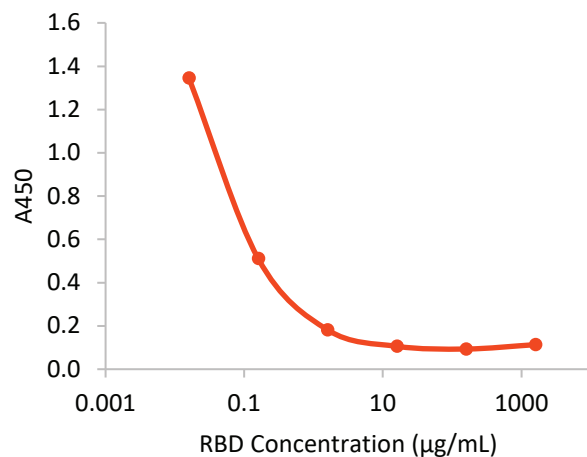
SDS-PAGE gel of the SARS-CoV-2 Spike Protein RBD expressed in HEK293 cells and purified with NGL COVID-19 Spike Protein Affinity Resin using a bind, wash, elute protocol.

### Experimental setup

- Feed: SARS-CoV-2 RBD
- Load: 1x PBS, pH 7, 6 min rt
- Wash 1: PBS, 5 CV, 0.5 CV/min
- Wash 2: PBS, 1 M NaCl, 5 CV, 0.5 CV/min
- Wash 3: PBS, 5 CV, 0.5 CV/min
- Elution: 100 mM citric acid, pH 2.0, 15 CV



Dynamic binding capacity (DBC) for SARS-CoV-2 Spike Protein RBD calculated at 10% breakthrough. Data was collected in two-minute intervals from 2 min to 10 min (average DBC at each time interval shown). DBC increased slightly as residence time was lengthened from 2 min to 6 min with no appreciable increase beyond 6 min.

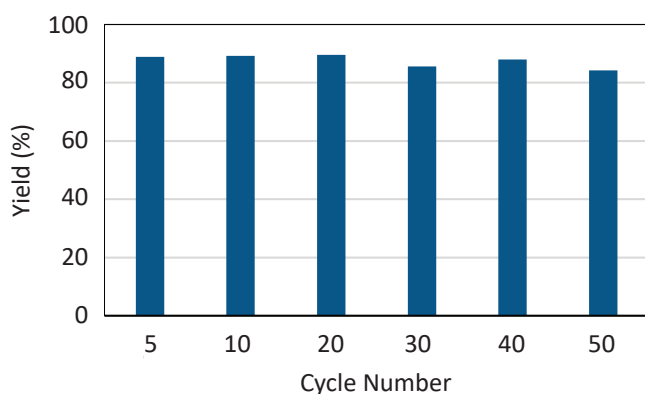


Binding of purified SARS-CoV-2 Spike Protein RBD to ACE-2 was assessed using an ELISA based inhibition assay. Serially diluted RBD was mixed with a fixed concentration of ACE-2 and then incubated in a well coated with RBD. The concentration of available ACE-2 was determined using an anti-ACE-2 secondary antibody colorimetric assay with detection at 450 nm. As the concentration of purified RBD increased, the amount of free ACE-2 available to bind to immobilized RBD decreased, demonstrating successful binding of RBD purified with the NGL COVID-19 Spike Protein Affinity Resin to its ACE-2 target.

# Implement in GMP Manufacturing

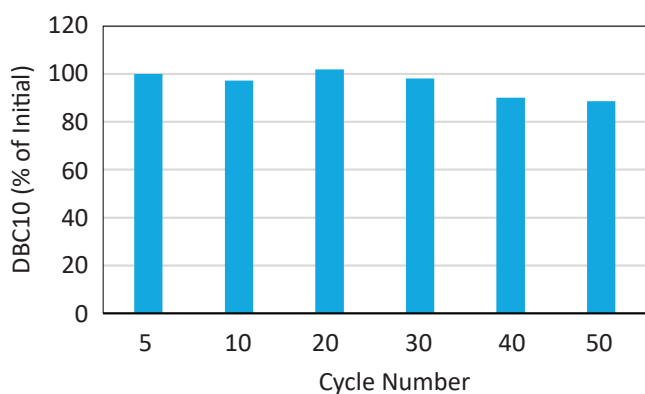
The NGL COVID-19 Spike Protein Affinity Resin meets GMP manufacturing expectations with high yield (> 85%) and dynamic binding capacity (DBC) (> 88%) sustained over multiple CIP and sanitization cycles. Receptor binding domain (RBD) clarified from CHO cell culture was purified over 50 complete cycles (load, wash, elute, CIP/sanitization) using NGL COVID-19 Spike Protein Affinity Resin. RBD yield and DBC were measured every fifth and tenth cycle, respectively.

### Consistent Yield across Cycles 1 - 50



RBD yield was measured every fifth cycle from 1 - 50 cycles (representative data shown). Yield remained consistent at 80 - 90% over the course of the study. Yield deviated by less than 3% (+/-) over 50 cycles of testing.

### > 88% Dynamic Binding Capacity after 50 Cycles



DBC for SARS-CoV-2 Spike Protein RBD was measured every tenth cycle from 1 to 50 cycles. Cycle 0 data was collected prior to initiating Cycle 1. DBC remained > 95% through Cycle 30 and > 88% through Cycle 50. DBC was calculated at 10% breakthrough.

Step	Buffer	CV
Equilibration buffer	PBS	5
Wash 1	PBS	3
Wash 2	PBS, 1 M NaCl	5
Wash 3	PBS	3
Elution	0.1 M acetic acid, pH 3.5	6
Cycle 1-4: CIP	0.05 M NaOH, 1 M NaCl	5
Cycle 5: Sanitization	0.1 M NaOH, 1% benzyl alcohol	5



# Specifications

## Product description

Characteristic	Description
Matrix composition	Highly cross-linked agarose
Ligand	Recombinant protein (E. coli expression)
Average particle size	85 µm
Coupling chemistry	Epoxy
Operational pressure	DO NOT EXCEED 1.5 bar deltaP
Operating temperature	4 - 30° C, Do not freeze
Delivery conditions	Shipped at room temperature, 52% slurry in 18% ethanol
Recommended pH	Operational: 3 - 10 Clean-in-Place (short term): 2 - 13
Storage conditions	18 - 20% ethanol or 2% benzyl alcohol
Storage temperature	2 - 8° C

## Product specifications

Specification	Value
Static binding capacity	≥ 15 mg SARS-CoV-2 RBD/ml resin
Dynamic binding capacity	> 15 mg/ml (RBD) at 6 min residence time*
Leachable ligand	≤ 50 ng ligand per ml resin

\* Dynamic binding capacity (DBC) was calculated at 10% breakthrough; actual DBC is dependent on S protein variant and size.

**NGL COVID-19 Spike Protein Affinity Resin is available in off-the shelf, pre-packed and pre-qualified OPUS® Columns for rapid implementation as well as in loose resin formats.**

Off-the-Shelf OPUS®			Made-to-Order OPUS®		Loose resin	
<b>OPUS® MiniChrom®</b>	<b>OPUS® ValiChrom®</b>	<b>OPUS®</b>	<b>OPUS®</b>	Your choice of OPUS® RoboColumn®, OPUS® MiniChrom®, OPUS® ValiChrom® and OPUS® 2.5 - 80R)	5 mL	10-SPIKE-0005
1 mL	100 mL	1 L (8 x 20)	33 L (45.7 x 20)		25 mL	10-SPIKE-0025
5 mL		2.5 L (12.6 x 30)	56 L (60 x 20)		100 mL	10-SPIKE-0100
		3 L (14 x 20)	100 L (80 x 20)		1 L	10-SPIKE-1000
		10 L (25 x 20)			5 L	10-SPIKE-5000

