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.

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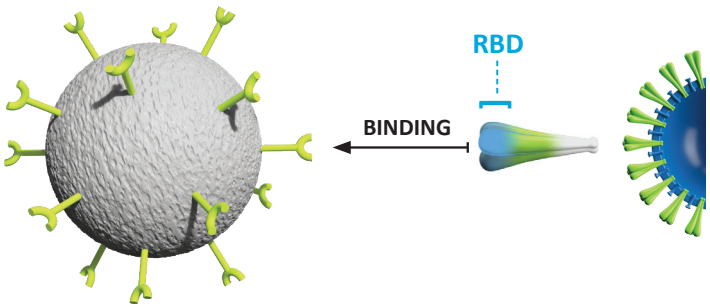
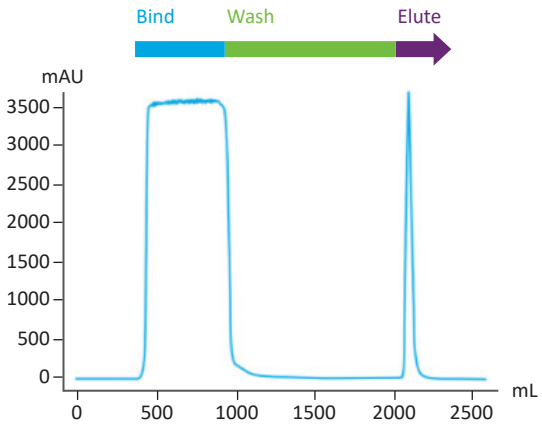
Navigo Proteins
PURE PRECISION

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



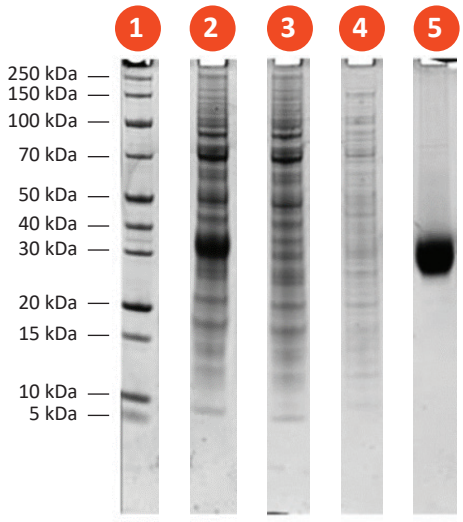
	OPUS® RoboColumn®	OPUS® MiniChrom®	OPUS® ValiChrom®	OPUS® 2.5 - 80R
Volume	50 µL - 600 µL	0.2 mL - 10 mL	2 mL - 300 mL	25 mL - 150 L
Column capacity*	750 µg - 9 mg	3 mg - 150 mg	30 mg - 4.5 g	375 mg - 2,250 g

* 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.



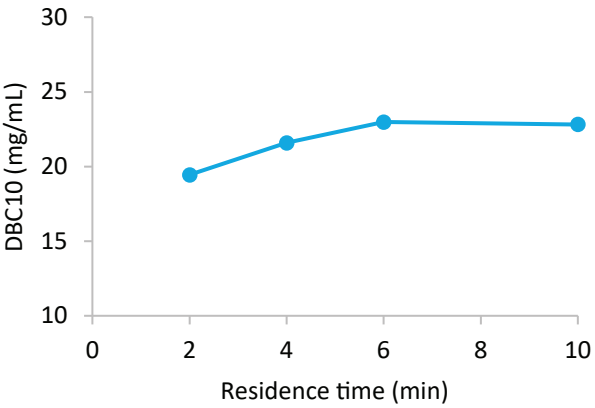
Lane	Sample
1	Marker
2	Load
3	Load FT
4	Wash
5	Elution

4 - 12% NuPage, 1X MES. Staining: Coomassie Blue. All samples reduced with 50 mM DDT.

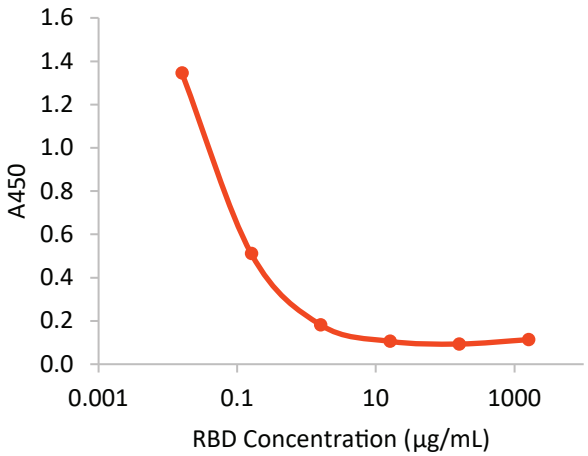
SDS-PAGE gel of the SARS-CoV-2 Spike Protein RBD expressed in HEK293 cells and purified with NGL COVID-19 Spike Protein Affinity Resin using a bind, wash, elute protocol.

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.

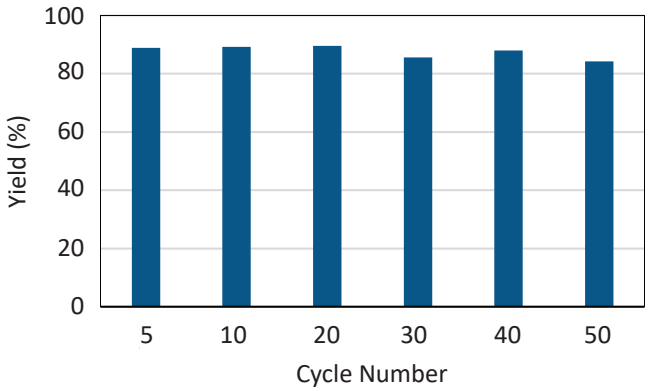


Binding of purified SARS-CoV-2 Spike Protein RBD to ACE-2 was assessed using an ELISA based inhibition assay. Serially diluted RBD was mixed with a fixed concentration of ACE-2 and then incubated in a well coated with RBD. The concentration of available ACE-2 was determined using an anti-ACE-2 secondary 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

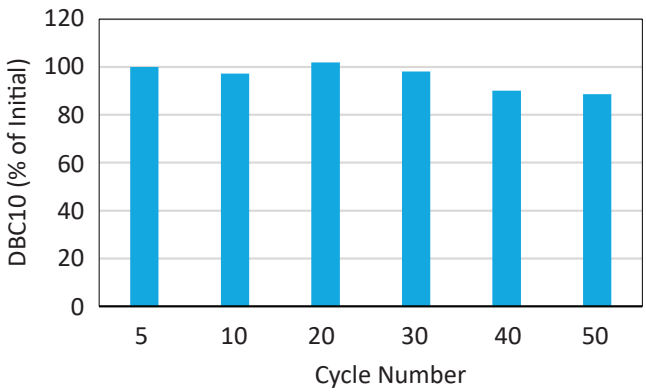
The NGL COVID-19 Spike Protein Affinity Resin meets GMP manufacturing expectations with high yield (> 85%) and dynamic binding capacity (DBC) (> 88%) sustained over multiple CIP and sanitization cycles. Receptor binding domain (RBD) clarified from CHO cell culture was purified over 50 complete cycles (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

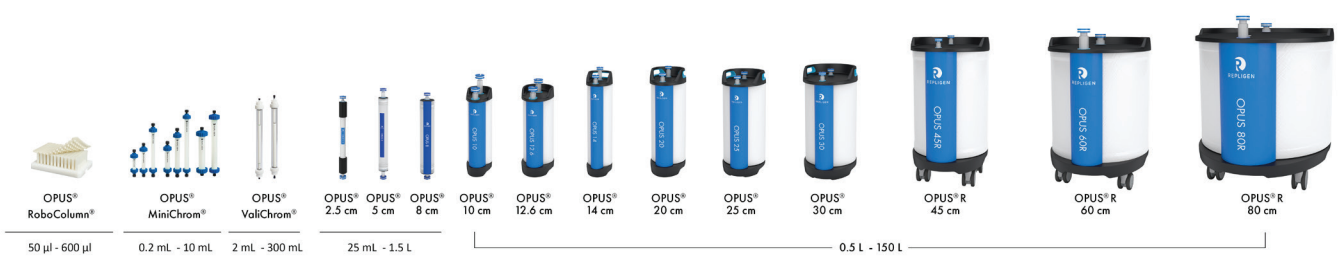
* Dynamic binding capacity (DBC) was calculated at 10% breakthrough; actual DBC is dependent on S protein variant and size.

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®		
OPUS® MiniChrom®	**OPUS® ValiChrom®**	**OPUS®**
1 mL	100 mL	1 L (8 x 20)
5 mL		2.5 L (12.6 x 30)
		3 L (14 x 20)
		10 L (25 x 20)

Made-to-Order OPUS®	
OPUS®	
33 L (45.7 x 20)	Your choice of OPUS® RoboColumn®, OPUS® MiniChrom®, OPUS® ValiChrom® and OPUS® 2.5 - 80R)
56 L (60 x 20)	
100 L (80 x 20)	

Loose resin	
5 mL	10-SPIKE-0005
25 mL	10-SPIKE-0025
100 mL	10-SPIKE-0100
1 L	10-SPIKE-1000
5 L	10-SPIKE-5000



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