NGL COVID-19 Spike Protein Affinity Resin

User Guide

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Abbreviations

A_s	Peak asymmetry
CF	Compression factor
CIP	Clean-in-Place
CV	Column volume
DBC	Dynamic binding capacity
EQ	Equilibration
GMP	Good manufacturing practice
HCP	Host cell protein
HETP	Height equivalent to a theoretical plate
NaCl	Sodium chloride
NaOH	Sodium hydroxide
NGL	Next Generation Ligand
PBS	Phosphate buffered saline
PPE	Personal protective equipment
RBD	Receptor binding domain
RT	Residence time

1. Introduction

The NGL COVID-19 Spike Protein Affinity Resin purifies SARS-CoV-2 Spike Protein receptor binding domain (RBD) variants to high 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 is available in off-the-shelf, pre-packed and pre-qualified OPUS® Columns for rapid implementation as well as in loose resin formats.

This user guide provides general guidance for the use of NGL COVID-19 Spike Protein Affinity Resin for purification of SARS-CoV-2 Spike Protein receptor binding domain variants. For further optimization or troubleshooting support, please contact your local Repligen Field Application Scientist (FAS). If you need assistance contacting your local FAS, the Customer Service team at Repligen would be happy to help (email: customerserviceUS@repligen.com; phone: 781-250-0111).

2. About this document

This manual uses several different phrases. Each phrase should draw the following level of attention:

Table 1. Explanation of user attention phrases

Phrase	Description
Note:	Points out useful information.
IMPORTANT	Indicates information necessary for proper instrument operation.
PRECAUTION	Cautions users of potential physical injury or equipment damage if the information is not heeded.
WARNING!	Warns users that serious physical injury can result if warning precautions are not heeded.

3. Safety precautions

Table 2. Safety precautions for NGL COVID-19 Spike Protein Affinity Resin

Symbol	Description
WARNING 	Wear standard laboratory personal protective equipment (PPE), including lab coat, protective eye wear, and gloves.
WARNING 	This product is for laboratory and manufacturing production use only. Not for administration to humans.
IMPORTANT 	This product is shipped in an 18.0 ±1% ethanol solution, a recognized bacteriostatic agent. It is flushed from the resin during equilibration and preparation for use. Follow all local regulations for safe disposal.
WARNING 	Flammable liquid and vapor. • Keep away from heat/spark/open flame/hot surfaces. No smoking. • Keep container tightly closed. • Ground/bond container and receiving equipment. • Store in a well-ventilated place. Keep cool.
IMPORTANT 	Dispose of contents/container in accordance with local/regional/national/ international regulations.
IMPORTANT 	For a full list of precautionary statements, please read the Safety Data Sheet (SDS).

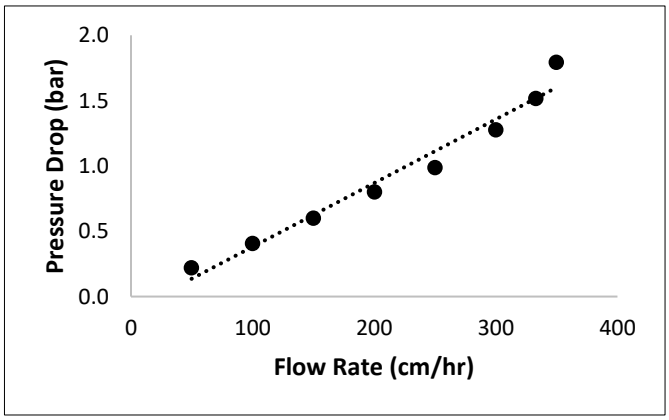
4. Product description

NGL COVID-19 Spike Protein Affinity Resin comprises an engineered affinity ligand immobilized to a cross-linked agarose support matrix. The NGL COVID-19 Spike Protein Affinity Resin enables a high purity capture step which decreases process time and improves overall yield in the production of COVID-19 vaccines.

Table 3. Product characteristics

Characteristics	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

Figure 1. Pressure-flow properties of NGL COVID-19 Spike Protein Affinity Resin



Pressure drop was measured with increasing linear flow rate from 50 - 400 cm/hr (Figure 1). NGL COVID-19 Spike Protein Affinity Resin was packed in an OPUS® 45R Column (45 cm D x 20 cm L). Compression factor: 1.2; mobile phase: 0.1 M NaCl.

5. Product specifications

Table 4. Product specifications

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

* DBC was calculated at 10% breakthrough; actual DBC is dependent on S protein variant and size.

6. Use recommendations

Table 5. Use recommendations

Use	Recommendation
Flow rate	Loading: ≥ 3 min residence time (RT); increase RT for larger molecules Equilibration (EQ)/ Wash: ≥ 2 min RT Elution/ CIP: ≥ 3 min RT Maximum flow rate (45 cm D X 20 cm L column): 300 cm/hr (4 min RT) Operational pressure: DO NOT EXCEED 1.5 bar deltaP
Loading pH	7.2-7.8
Equilibration and wash buffer	Phosphate buffered saline (PBS), pH 7.2-7.8 Wash buffer composition and volume may require optimization Additional secondary wash buffers may be used to improve HCP clearance: • PBS, 1 M NaCl • 20 mM sodium citrate, pH 5.5-6.0
Elution buffer	0.1 M acetic acid, pH 3.5 Alternative elution buffer(s): • 100 mM sodium acetate, pH 4.0 + 1 M arginine • 100 mM sodium acetate, pH 4.3 + 2 M $MgCl_2$
Strip	200 mM acetic acid (upflow recommended)
CIP*	CIP (upflow recommended) Five (5) cycle cadence example: • Cycle 1-4: 0.05 M NaOH, 15-30 min contact time 1 M NaCl • Cycle 5: 0.1 M NaOH, 15 min contact time * Cleaning recommendations dependent on feedstock
Storage solution	18-20% ethanol or 1-2% benzyl alcohol

Table 6. General protocol

Step	Buffer	Residence time	CV
Equilibration	PBS	≥ 2 min	5
Load	Spike protein, pH 7.2-7.8	≥ 3 min	-
Wash 1	PBS	≥ 2 min	3
Wash 2	PBS, 1 M NaCl	≥ 2 min	5
Wash 3	PBS	≥ 2 min	3
Elution	0.1 M acetic acid, pH 3.5	≥ 3 min	3 - 5
Strip	200 mM acetic acid	≥ 3 min	3
CIP cycle 1-4	0.05 M NaOH, 1 M NaCl	≥ 3 min (15-30 min contact)	3
CIP cycle every 5th	0.1 M NaOH	≥ 3 min (15 min contact)	3

*Flow rate limits will depend on column geometry, DO NOT EXCEED 1.5 bar deltaP.

7. Column packing instructions

- The resin is supplied as 52% slurry in 18% ethanol. In preparation of column packing, exchange the shipping solution with 0.1 M sodium chloride. Alternatively, phosphate buffered saline (PBS) can be used as the packing buffer. The resin may be packed with flow pack or axial compression methods.

7.1 Flow packing

1. Decant storage solution and re-suspend resin in the desired packing buffer.
2. Attach bottom flow adaptor to column body.
3. Transfer the resin slurry into the column. Take into account a target compression factor (CF) of 1.2 in order to achieve the desired final column volume (CF of 1.1-1.3, depending on the packing pressure).
4. Close the outlet port of the column and connect the top flow adapter to the tubing of the three-way valve labeled “To Column”.
5. Set the three-way valve to the *pump to purge* position and prime the flow path.
6. Set the three-way valve to the *column to purge* position. Lower the adapter into the column and allow liquid to vent through the three-way valve purge line until air is purged.
7. Lock flow adapter in place. Set the three-way valve to the *pump to column* position. Open the bottom port of the column and flow at 100 cm/hr until bed has formed. Stop the pump. Close the bottom column port.
8. Set the three-way valve to the *column to purge* position. Manually lower the flow adaptor until it reaches 0.5 - 1 cm above the settled bed. Liquid should purge through the top of the column via the three-way valve.
9. Set the three-way valve to the *pump to column* position. Open the bottom column port and flow buffer up to a pressure of 1.5 bar deltaP and mark the top of the resin bed once stabilized. Maintain flow for a minimum of 3 CV. Stop the pump. Close the bottom column port.
10. Set the three-way valve to the *column to purge* position and manually lower the flow adapter to the bed height marked in the previous step.
11. Set the three-way valve to *pump to column* position. Open the bottom column port. Flow buffer for 3 CV at a flow rate that creates 1.5 bar to condition the column. If a gap forms between the flow adaptor and the bed, lower the adapter and repeat the previous step. Do not run at the packing pressure again or the column will continue packing down.

12. Evaluate column performance using HETP and asymmetry.
    a. (HETP) > 2,000 N/m
    b. Peak asymmetry 0.8-1.8

Note: *If HETP is <2000 N/m and asymmetry >1.8, increase compression factor by lowering the flow adapter in 0.02 CF increments and re-test. If asymmetry <0.8, reduce compression in 0.02 CF increments and re-test.*

7.2 Axial Compression

1. Decant storage solution and re-suspend resin in the desired packing buffer. The recommended packing buffer is 0.1 M NaCl, Alternatively, phosphate buffered saline (PBS) or water can be used as the packing buffer.
2. Attach bottom flow adaptor to column body.
3. Transfer the resin slurry into the column. Take into account a target compression factor (CF) of 1.2 in order to achieve the desired final column volume (CF of 1.1-1.3, depending on the packing pressure).
4. Close the outlet port of the column and connect the top flow adapter to the tubing of the three-way valve labeled “To Column”.
5. Set three-way valve to the *pump to purge* position and prime the flow path. Close the outlet port of the column and connect the flow adapter to the top of the column.
6. Set the three-way valve to the *column to purge* position. Lower the adapter into the column and allow liquid to vent through the top port until air is purged.
7. Lock flow adapter in place. Set the three-way valve to the *pump to column* position. Open the bottom port of the column and flow at 100 cm/hr until bed has formed. Stop the pump.
8. With the pump stopped, keep the three-way valve in the *pump to column* position and the bottom outlet port open, lower the flow adaptor at a rate of 150 cm/hr until the target compression factor is achieved (liquid will flow out of the bottom port of the column).
9. Flow condition the column with an additional 3 CVs packing buffer at a flow rate that achieves 1.5 bar. If a gap forms between the flow distributor and the bed, lower the adapter and repeat flow condition step above. Do not run at the packing pressure again or the column will continue packing down.
10. Evaluate column performance using HETP and asymmetry.
    a. (HETP) > 2,000 N/m
    b. Peak asymmetry 0.8-1.8

Note: *If HETP is <2000 N/m and asymmetry >1.8, increase compression factor by lowering the flow adapter in 0.02 CF increments and re-test. If asymmetry <0.8, reduce compression in 0.02 CF increments and re-test.*

8. Column qualification

Column qualification is typically determined by testing HETP (height equivalent to a theoretical plate) and A_s (peak asymmetry).

IMPORTANT: For best results, avoid sample dilution by applying the sample as close to the column inlet as possible, and placing the conductivity meter as close to the column outlet as possible.

Table 7. Recommended column efficiency testing parameters

Condition	Recommendation
Detection	Conductivity
Effluent solution	0.1 M NaCl
Sample volume	1% of the column volume
Sample concentration	1 M NaCl
Flow rate	100 cm/hr

9. Ordering information

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.

More information regarding OPUS® pre-packed chromatography columns can be found by visiting https://www.repligen.com/technologies/opus.

Table 8. Part numbers for NGL COVID-19 Spike Protein Affinity Resin

Resin volume	Part number
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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