

NGL COVID-19 Spike Protein AR 2.0

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

NGL COVID-19 Spike Protein AR 2.0 Resin purifies SARS-CoV-2 Spike Protein and sub-units containing the receptor binding domain (RBD) 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, dynamic binding capacity, and caustic stability over multiple cycles.

NGL COVID-19 Spike Protein AR 2.0 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 AR 2.0 Resin for purification of SARS-CoV-2 Spike Proteins and sub-units containing the RBD. 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 user attention 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 AR 2.0 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. <ul style="list-style-type: none"> 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 AR 2.0 Resin comprises an engineered affinity ligand immobilized to a cross-linked agarose support matrix. The NGL COVID-19 Spike Protein AR 2.0 Resin enables a high purity capture step which decreases process time and improves overall yield in the production of COVID-19 spike protein-based vaccines.

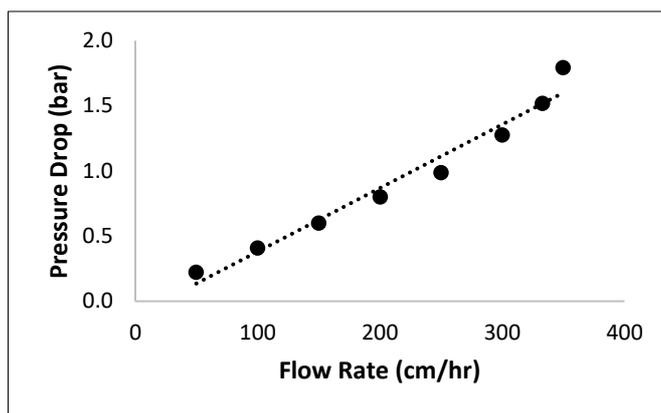
Table 3. Product characteristics

Characteristics	Description
Matrix composition	Highly cross-linked agarose
Ligand	Recombinant protein (<i>E. coli</i> 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 50% 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

Table 4. Product Variant Binding

SARS-CoV-2 Variant	NGL COVID-19 Spike Protein Affinity Resin	NGL COVID-19 Spike Protein AR 2.0 Resin
Wild Type	+++	+++
Alpha	+++	+++
Beta	-	+++
Gamma	-	+++
Delta	+++	+++
Delta +	+++	+++
Kappa	+++	+++
Lambda	+++	+++
Omicron	+	+

Figure 1. Pressure-flow properties of NGL COVID-19 Spike Protein AR 2.0 Resin



Pressure drop was measured with increasing linear flow rate from 50 - 400 cm/hr (Figure 1). NGL COVID-19 Spike Protein AR 2.0 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.

Table 5. Product specifications

Specification	Value
Static binding capacity	≥ 10 mg SARS-CoV-2 RBD/ml resin
Dynamic binding capacity	> 10 mg SARS-CoV-2 RBD/ml resin at 6 min residence time ¹
Leachable ligand	≤ 50 ng ligand per ml eluant

¹DBC was calculated at 10% breakthrough; actual DBC is dependent on Spike protein variant and size.

Table 6. 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
Elution buffer	0.1 M acetic acid, pH ≤ 4.0 Alternative elution buffer: <ul style="list-style-type: none"> • 100 mM sodium acetate, pH 5.5 + 1 M arginine
Strip	200 mM acetic acid
CIP	CIP (upflow recommended) <ul style="list-style-type: none"> • 0.1 M NaOH, 15 min contact time Cleaning recommendations are dependent on feedstock
Storage solution	18 - 20% ethanol or 1 - 2% benzyl alcohol

Table 7. General protocol

Step	Buffer	Residence time	CV
Equilibration	PBS	≥ 2 min	5
Load	Spike protein, pH 7.2 - 7.8	≥ 3 min	-
Wash	PBS	≥ 2 min	5-10
Elution	0.1 M Sodium Acetate, pH 4.0	≥ 3 min	3 - 5
Strip	200 mM acetic acid	≥ 3 min	3
CIP	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.

5. Column packing

The resin is supplied as an approximate 50% slurry in 18% ethanol. Repligen recommends performing a percent slurry measurement prior to column dosing for accurate results. Recommended packing buffer is 0.1 M sodium chloride or phosphate buffered saline (PBS). Prior to packing the shipping solution should be exchanged with packing buffer. The resin may be packed using flow pack or axial compression.

Table 8. Column Packing Parameters

Parameter	Value
Compression Factor	1.2 (1.1 - 1.3)
Consolidation Flow Velocity (cm/hr)	100
Packing Pressure	≤ 1.5 bar
Packing CV	≥ 3 CV
Conditioning Pressure	1.5 bar
Conditioning CV	≥ 3 CV

5.1 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 9. 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
HETP (N/m)	≥ 2000
Asymmetry	0.8 - 1.8

6. Ordering information

NGL COVID-19 Spike Protein AR 2.0 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 10. Part numbers for NGL COVID-19 Spike Protein AR 2.0 Resin

Resin volume	Part number
5 mL	SPIKE-AR20-0005
25 mL	SPIKE-AR20-0025
100 mL	SPIKE-AR20-0100
1 L	SPIKE-AR20-1L
5 L	SPIKE-AR20-5L

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