ELISA Kit performance summary: NGL COVID-19 Spike Protein Affinity Ligand in the presence of SARS-CoV-2 spike protein RBD

Introduction

The NGL COVID-19 Spike Protein Affinity Ligand ELISA Kit (part number 9444-1) from Repligen provides accurate and precise quantitation of residual NGL COVID-19 Spike Protein Affinity Ligand in SARS-CoV-2 spike protein products purified with NGL COVID-19 Spike Protein Affinity Resin.

Testing for residual NGL COVID-19 Spike Protein Affinity Ligand occurs in several different phases of development and commercial manufacturing which may include:

- Process development: leaching characteristics of the resin under specific conditions
- Manufacturing: eluted samples taken throughout several points in the purification process
- Finish product release: document process containment levels and lot-to-lot consistency

ELISA Kits from Repligen are thoroughly validated to help ensure they perform as expected and consistently generate accurate and reproducible data. All kits are evaluated for the following factors:

- **Intra-assay precision:** ensures reproducible results from well-to-well within the same plate
- **Inter-assay precision:** ensures reproducible results from plate-to-plate
- **Accuracy:** measured as % recovery, demonstrates analyte detection is not affected by differences in sample matrix (e.g., cell culture media, Spike Protein)
- **Limit of detection (LOD):** lowest quantity or concentration of an analyte that can be reliably distinguished from background
- **Limit of quantitation (LOQ):** lowest concentration at which an analyte can be quantified

The performance of NGL COVID-19 Spike Protein Affinity Ligand ELISA Kit was evaluated when detecting the NGL COVID-19 Spike Protein Affinity Ligand in the presence of SARS-CoV-2 receptor binding domain (RBD) compared to a standard containing no RBD. All spiked samples had a final RBD concentration of 0.125 mg/mL (following final dilution into the assay plate). Each sample was prepared in triplicate; three (3) separate ELISAs were performed according to the kit's standard protocol. For additional details, refer to the Section Explanation of Calculations.

Intra-assay precision

Average %CV (coefficient of variation) data for both standard curve samples without RBD (Table 1) and standard curve samples with RBD (Table 2) demonstrate intra-assay reproducibility. For standard curve samples without RBD, average %CV ranged from 3.2 - 5.2%. For standard curve samples with RBD, average %CV values ranged from 1.1 – 3.5%.

Table 1. Intra-assay precision: standard curve samples without RBD

Concentration (ng/mL)	Calculated conc. (ng/mL)	Avg %CV
10	10.03	3.2%
5	4.97	3.4%
2.5	2.56	3.8%
1.25	1.31	3.1%
0.625	0.64	5.2%
0.3125	0.30	5.2%

Table 2. Intra-assay precision: standard curve samples with RBD

Concentration (ng/mL)	Calculated conc. (ng/mL)	Avg %CV
5	4.92	3.5%
2.5	2.20	1.1%
1.25	1.14	2.3%

Inter-assay precision

Average %CV data for both standard curve samples without RBD (Table 3) and standard curve samples with RBD (Table 4) demonstrate inter-assay reproducibility. For standard curve samples without RBD, %CV was less than 2.4% for all samples. For standard curve samples with RBD, %CV was less than 10.7% for all samples.

Table 3. Inter-assay precision: standard curve samples without RBD

Concentration (ng/mL)	Calculated conc. (ng/mL)	Avg %CV
10	10.03	0.1%
5	4.97	2.4%
2.5	2.56	0.4%
1.25	1.31	1.5%
0.625	0.64	1.2%
0.3125	0.30	1.6%

Table 4. Inter-assay precision: standard curve samples with RBD

Concentration (ng/mL)	Calculated conc. (ng/mL)	Avg %CV
5	4.92	7.1%
2.5	2.20	4.7%
1.25	1.14	10.7%

Accuracy

Gauged by % relative error, accuracy ranged from -4.7% to 4.5% for standard curve samples without RBD (Table 5) and from 1.6% to 12.2% for standard curve samples with RBD (Table 6).

Table 5. Accuracy: standard curve samples without RBD

Concentration (ng/mL)	Average % Error	Avg % Recovery
10	0.3%	100.3%
5	-0.5%	99.5%
2.5	2.4%	102.4%
1.25	4.5%	104.5%
0.625	2.9%	102.9%
0.3125	-4.7%	95.3%

Table 6. Accuracy: standard curve samples with RBD

Concentration (ng/mL)	Average % Error	Avg % Recovery
5	1.6%	98.4%
2.5	12.2%	87.8%
1.25	8.8%	91.2%

Accuracy of dilution

Accuracy of dilution was evaluated using the Dilute & Go method of the ELISA protocol. Sample concentration was plotted versus absorbance at 450 nm. Quadratic regression analysis demonstrates accurate measurement of ligand concentration across the assay's dynamic range (Figure 1).

For standard curve samples without RBD, the limit of quantitation (LOQ) was 0.07 ng/mL and the limit of detection (LOD) was 0.20 ng/mL. For standard curve samples with RBD, the LOQ was 0.56 ppm and the LOD was 1.60 ppm.

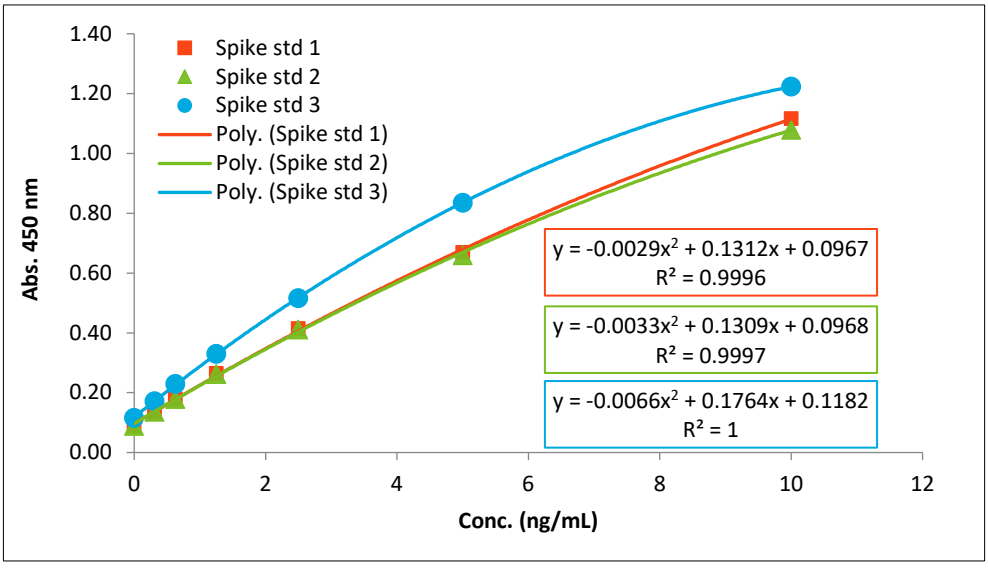

Figure 1. Accuracy of dilution by Dilute & Go method

Conclusions

The performance metrics outlined in this Performance Summary demonstrate that the NGL COVID-19 Spike Protein Affinity Ligand ELISA Kit provides accurate and precise quantitation of residual NGL COVID-19 Spike Protein Affinity Ligand in SARS-CoV-2 spike protein products purified with NGL COVID-19 Spike Protein Affinity Resin.

Explanation of calculations

Accuracy
Accuracy is described as the % recovery determined by the assay compared to the theoretical spiked concentration.

Accuracy of dilution
Sample concentration was plotted versus absorbance at 450 nm. Accuracy of dilution was assessed using regression analysis with a quadratic fit.

Inter-assay precision
The inter-assay precision was calculated for each concentration point by determining the standard deviation between calculated results from each of the three assays, then dividing by the mean value.

Intra-assay precision
The intra-assay precision was calculated for each RBD spiked sample concentration by averaging the %CV values across all assays.

Limit of detection (LOD)
The limit of detection (LOD) was defined as 3 times the standard deviation of 0 ng/ml NGL COVID-19 Spike Protein Affinity Ligand sample. The standard deviation of the 0 ng/ml OD value was multiplied by 3 then added to base 0 ng/ml OD value. The LOD was then generated by entering the summed value into the standard curve equation. For each kit a LOD was reported as ng NGL COVID-19 Spike Protein Affinity Ligand per mL (ng/mL) buffer and in parts per million (ppm).

Limit of quantitation (LOQ)
The limit of quantitation (LOQ) was defined as 10 times the standard deviation of 0 ng/mL sample. The standard deviation of the 0 ng/mL OD value was multiplied by 10 then added to base 0 ng/mL OD value. The LOQ was then generated by entering the summed value into the standard curve equation. For each kit the LOQ was reported as ng NGL COVID-19 Spike Protein Affinity Ligand per ml (ng/mL) buffer, and ng NGL COVID-19 Spike Protein Affinity Ligand per mg RBD (ppm) for RBD spiked samples run in presence of RBD (ppm).

Percent recovery
% Recovery = [Calculated Concentration / Theoretical Concentration] x 100.

Precision (%CV)
Precision was calculated by determining the standard deviation between RBD spiked sample data points and dividing by the mean value. According to the Guidance for Industry: Bio-analytical Method Validation text, precision should be within 15%.