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**Application Note: Use of A02161 as positive control for L00847-A
SARS-CoV-2 Surrogate Virus Neutralization Test (sVNT) Kit**

Description of SARS-CoV-2 (Omicron) Neutralizing Antibody Standard (Cat. No.: A02161)

SARS-CoV-2 (Severe acute respiratory syndrome coronavirus 2) is a positive-sense single-stranded RNA virus. It caused coronavirus disease 2019 (COVID-19). The B.1.1.529 variant was first reported to WHO from South Africa on 24 November 2021. WHO has designated B.1.1.529 as a VOC, named Omicron. SARS-CoV-2 contains glycosylated spike (S) protein, which is composed of S1 subunit and S2 subunit. The S1 contains a receptor-binding domain (RBD) that can bind to ACE2 receptor on target cells. Neutralizing antibodies against SARS-CoV-2 can block the interaction between SARS-CoV-2 RBD and ACE2. The blocking ability of SARS-CoV-2 neutralizing antibodies is affected by the quantity.

This standard product is intended for the calibration of SARS-CoV-2 Omicron neutralizing antibodies. It can be used in the assessment and development of assays for the detection and quantitation of SARS-CoV-2 Omicron neutralizing antibodies. The neutralizing antibody standard binds to multiple neutralizing epitopes in the receptor-binding domain. The product can recognize and neutralize Wild-Type SARS-CoV-2 and five Variants of Concern (VOC) including Alpha, Beta, Gamma, Delta, and Omicron. The concentration of this product is 800,000 U/ml.

A02161 as the positive control when Omicron RBD-HRP (Z03730) is used with SARS-CoV-2 Surrogate Virus Neutralization Test (sVNT) Kit (GenScript, Cat.No L00847-A)

Users can design assays to detect SARS-CoV-2 neutralizing antibodies against Omicron RBD using Omicron RBD-HRP (Z03730) with the sVNT kit. Due to the immune escape feature of the Omicron variant,¹⁻³ significant reduction in neutralization was observed when using the Omicron RBD-HRP (Z03730) with the positive control supplied in sVNT.

Results from in-house testing

	OD450 (Z03730 Omicron RBD-HRP)
L00847-Positive Control (PC)	1.2249
L00847-Negative Control (NC)	2.3246

As A02161 can neutralize the omicron variant, it can be used as the positive control when Omicron RBD-HRP (Z03730) is used with sVNT.

Recommended working concentration of A02161 when used as positive control for sVNT

It is recommended to use 80,000 U/ml and above when A02161 is used as positive control in sVNT. Based on the data below, this will generate an average OD450 of 0.1492 with a standard deviation of 0.0270.

Results from in-house testing

Working concentration of A02161 (U/ml)	Run 1		Run 2		Run 3	
	OD450	% inhibition	OD450	% inhibition	OD450	% inhibition
100,000	0.1307	94.42%	0.1303	94.25%	0.1695	93.92%
80,000	0.1314	94.39%	0.1288	94.32%	0.1873	93.29%
60,000	0.1629	93.05%	0.1545	93.18%	0.2217	92.05%

Note: The working concentration in the table is to be subjected to 10-fold dilution by sample dilution buffer, and further 2-fold dilution by RBD-HRP solution as per L00847-A manual.

References

- 1 Wilhelm, A. et al. Reduced Neutralization of SARS-CoV-2 Omicron Variant by Vaccine Sera and Monoclonal Antibodies. medRxiv, 2021.2012.2007.21267432 (2021).
- 2 Cele, S. et al. SARS-CoV-2 Omicron has extensive but incomplete escape of Pfizer BNT162b2 elicited neutralization and requires ACE2 for infection. medRxiv, 2021.2012.2008.21267417 (2021).
- 3 Sokal, A. et al. Immune escape of SARS-CoV-2 Omicron variant from mRNA vaccination-elicited RBD-specific memory B cells. bioRxiv, 2021.2012.2021.473528 (2021).

Caution

For Research Use Only. Not for Use in Diagnostic Procedures.