Data Sheet

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GenScript cPass® SARS-CoV-2 Neutralization Antibody Detection Kit

For the detection of human neutralizing antibodies to the SARS-CoV-2 S1 RBD protein from:

– Human serum

– Human plasma

Introduction

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is the causative agent for the highly contagious coronavirus disease. Infection with the SARS-CoV-2 virus triggers an immune response within the body which in turn produces antibodies. These secreted antibodies may help to protect the individual against future infections. A sub-population of these generated antibodies, called neutralizing antibodies have been demonstrated in a lab to block the virus from infiltrating the cell [1-6].

The GenScript cPass® SARS-CoV-2 kit is a blocking ELISA detection kit that can help to detect generated neutralizing antibodies in patient samples that prevent binding of the receptor binding domain (RBD) of the spike virus to the angiotensin converting enzyme-2 (ACE-2) of the host cell receptor [7]. It is intended for use as an aid in identifying individuals with an adaptive immune response to SARS-CoV-2, indicating recent or prior infection. The cPass® SARS-CoV-2 Neutralization Antibody Detection kit should not be used to diagnose or exclude acute SARS-CoV-2 infection.

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Order Information

Product Name	Part Number
GenScript cPass® SARS-CoV-2 Neutralization Antibody Detection kit	L00847

Three Key Criteria

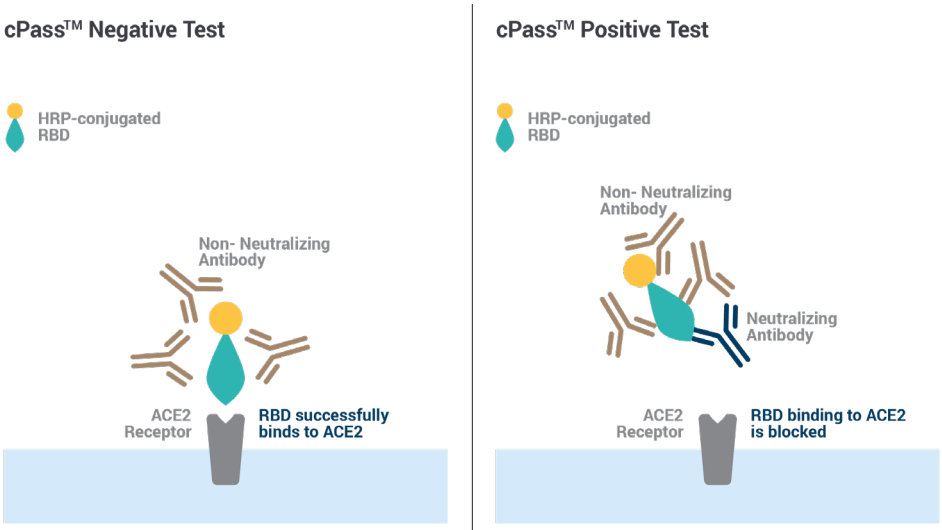
- High specificity/sensitivity, spike protein as a target (increases accuracy)
- Neutralizing antibodies are an important biomarker for possible immunity; presence of binding antibodies may not guarantee presence of neutralizing antibodies and levels may not be consistent.
- Population testing may help decision makers ultimately understand risks, trends, and patterns in subpopulations and may aid in making further decisions regarding booster doses and vaccine efficacy

Characteristics

Number of Tests per Kit	Samples: 92 tests Positive control: 2 tests Negative control: 2 tests
Sample Type	Human Serum Human Plasma
Antibody Class	Human neutralizing antibodies
Protein Target	Spike protein (RBD) of SARS-CoV-2 virus
Assay Packaging	1 standard ready-to-use 96-well pre-coated microplate Ready-to-use kit with all reagents including negative and positive controls
Incubation Temperatures	37°C after addition of neutralization reaction mixture to ACE-2 coated assay plates 25°C after addition of TMB solution
Test Time	< 2 hours
Interpretation of Results	≥ 30%, Positive, SARS-CoV-2 neutralizing antibody detected < 30%, Negative, no detectable SARS-CoV-2 neutralizing antibody The cut-off value refers to 30% Signal Inhibition

Performance

	Using Plaque Reduction Neutralization test ($PRNT_{50}$) as a comparator	Using RT-PCR as a Comparator
Sensitvity	100% (Study 1 with 114 samples) and 95.7% (Study 2 with 140 samples)	94.0% (95% CI 88.6-100.0%)
Specificity	100% (Study 1 with 114 samples and 97.8% (Study 2 with 140 samples)	96.5% (95% CI 90.2-98.8%)
Number of Positive Samples	Study 1: 26 Study 2: 45	133
Number of Negative Samples	Study 1: 88 Study 2: 91	86



If only binding antibodies are present in the sample, the HRP labeled RBD still binds to the ACE-2 receptor on the plate and generates strong signal.

If neutralizing/blocking antibodies are also present in the sample, they would bind to some of the HRP-RBD conjugate and prevent it from binding to the ACE-2 receptor on the plate. During the wash step the blocked HRP-RBD is removed, therefore decreasing the signal detected in the well.

Figure 1. cPass® SARS-CoV-2 kit is blocking ELISA detection kit that can help to detect generated neutralizing antibodies in patient samples that prevent binding of the receptor binding domain (RBD) of the spike virus to the angiotensin converting enzyme-2 (ACE-2) of the host cell receptor[7].

Analytical Specificity (Cross-Reactivity)

Cross-reactivity of cPass® SARS-CoV-2 neutralization antibody kit was evaluated using a total of 60 SARS-CoV-2 seronegative patient specimens. These 60 samples (2 replicates) tested seropositive for various viral diseases. Cross reactivity is only found with SARS-CoV-1. It is possible however that patients infected with SARS-CoV-1 may have neutralizing antibodies that are cross functional to SARS-CoV-2. This needs further studies (see Table 1). No cross-reactivity was observed with any of the hCoV sera tested nor any of the other anti-sera tested in this study.

Table 1. Analytical specificity: cross-reactivity of cPass® SARS-CoV-2 neutralization antibody kit.

Disease	Number of Samples (n)	Number of False Positive	Number of False Negative
Human Coronavirus OC43	2	0	2
Human Coronavirus 229E	2	0	2
Dengue Virus	3	0	3
Zika Virus	1	0	1
Mers-CoV (Alpaca)	2	0	2
Influenza A/B	11	0	11
HCV	5	0	5
ANA	5	0	5
RSV	7	0	7
HBV	10	0	10
HIV	10	0	10
SARS-CoV-1	2	2	0
	60	2	58
	Specificity: 96.7%		

Clinical Study

In order to validate the clinical performance of the GenScript cPass® SARS-CoV-2 Neutralization Antibody Detection kit, the comparator Plaque Reduction Neutralization Test (PRNT) utilizing the SARS-CoV-2 virus (WA01/2020 isolate) was used. The cutoff for the PRNT comparator tests was established as indicated in Table 2.

Table 2. Cut-off specifications for PRNT tests

	Value Result (Dilution titer)	Result	Total Result Interpretation
$PRNT_{50}$	≥ 1:20	Positive	Neutralizing antibodies for SARS-CoV-2 are detected at 50% viral neutralization
	≤ 1:20	Negative	Neutralizing antibodies for SARS-CoV-2 are not detected at 50% viral neutralization
$PRNT_{90}$	≥ 1:10	Positive	Neutralizing antibodies for SARS-CoV-2 are detected at 90% viral neutralization
	≤ 1:10	Negative	Neutralizing antibodies for SARS-CoV-2 are not detected at 90% viral neutralization

The first clinical agreement study evaluated a total of 114 samples retrospectively collected from SARS-CoV-2 RT-PCR positive and negative individuals (26 PRNT positive and 88 PRNT negative) using the cPass® SARS-CoV-2 Neutralization Antibody Detection kit and the PRNT comparator ($PRNT_{50}$ and $PRNT_{90}$). The combined cohort consisted of samples from normal healthy people (n=88) and samples from RT-PCR confirmed SARS-CoV-2 positive patients (n=26). The GenScript cPass® SARS-CoV-2 Neutralization Antibody Detection kit sample results were compared to a Plaque Reduction Neutralization test performed to WHO guidelines. The following Table 3 show the Positive and Negative Percent Agreement between the $PRNT_{50}$ or $PRNT_{90}$ and the cPass® SARS-CoV-2 Neutralization Antibody Detection kit results.

Table 3. Results of cPass® SARS-CoV-2 Neutralization Antibody Detection kit sample results compared to a Plaque Reduction Neutralization test (Study 1).

		Positive	Negative	Positive Percent Agreement	Negative Percent Agreement
Plaque Reduction Neutralization test ($PRNT_{50}$)	Positive (n=26)	26	0	100% (95% CI 87.1-100.0%)	
	Negative (n=88)	0	88		100% (95% CI 95.8-100.0%)

The second clinical agreement study evaluated a total of 140 samples retrospectively collected from SARS-CoV-2 RT-PCR positive individuals using the cPass® SARS-CoV-2 Neutralization Antibody Detection kit. The cohort consisted of 93 $PRNT_{50}$ negative samples and 47 $PRNT_{50}$ positive samples. The cPass® SARS-CoV-2 Neutralization Antibody Detection kit results were compared to a $PRNT_{50}$ comparator test. Overall PPA and NPA are shown in Table 4.

Table 4. Results of cPass® SARS-CoV-2 Neutralization Antibody Detection kit sample results compared to a Plaque Reduction Neutralization test (Study 2).

		Positive	Negative	Positive Percent Agreement	Negative Percent Agreement
Plaque Reduction Neutralization test ($PRNT_{50}$)	Positive (n=47)	45	2	95% (95% CI 85.8-98.8%)	
	Negative (n=93)	2	91		97.8% (95% CI 92.5-99.4%)

References

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The sensitivity of cPass® SARS-CoV-2 Neutralization Antibody Detection kit early after infection is unknown. Negative results do not preclude acute SARS-CoV-2 infection.
If acute infection is suspected, direct testing for SARS-CoV-2 is necessary.
False positive results for the cPass® SARS-CoV-2 Neutralization Antibody Detection kit may occur due to cross-reactivity from pre-existing antibodies or other possible causes.

The cPass® SARS-CoV-2 Neutralization Antibody Detection kit has been authorized by FDA under an EUA for use by authorized laboratories of in vitro diagnostics for qualitative detection of total neutralizing antibodies to SARS-CoV-2 in human serum and K2-EDTA plasma in laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C 263a that meet requirements to perform high complexity tests.

For technical questions relating to cPass® SARS-CoV-2 Neutralization Antibody Detection kit, please contact us at covid.support@agilent.com

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