SARS (Coronavirus) and MERS Antigens

ZIVA™ Acute Respiratory Viral Serology Array

For research use only. Not for use in diagnostic procedures

ADARZA BIOSYSTEMS

Description

SARS and MERS Coronavirus antigens

Strain	Region	Uniprot Accession Number
HCoV-HKU1	Spike S1 Protein	Q5MQD0
HCoV-229E	Spike S1 + S2 Protein	P15423
HCoV-OC43	Spike S1 + S2 Protein	P36334
HCoV-NL63	Spike S1 Protein	Q6Q1S2
SARS-CoV-2 (2019-nCoV)	Spike S1 (D614G)-His	P0DTC2
SARS-CoV-2 (2019-nCoV)	Spike S1-His	P0DTC2
SARS-CoV-2 (2019-nCoV)	Spike RBD-His	P0DTC2
SARS-CoV-2 (2019-nCoV)	Nucleocapsid-His	P0DTC9
SARS-CoV-2 (2019-nCoV)	Spike S1+ S2 ECD-His	P0DTC2
SARS-CoV-2 (2019-nCoV)	Spike S2 ECD-Fc	P0DTC2
MERS-CoV	Spike S1 Protein	K0BRG7
MERS-CoV	Spike RBD Protein	K0BRG7
MERS-CoV	Nucleocapsid Protein	K0BVN3
SARS-CoV	Spike S1 Protein	Q5DIC5
SARS-CoV	Spike RBD Protein	Q5DIC5
SARS-CoV	Nucleocapsid Protein	P59595

Regions of the common coronaviruses are analyzed within this panel. Coronaviruses consist of the following main proteins: the spike protein, the envelope protein, the membrane protein, and the nucleocapsid protein. Coronaviruses are enveloped viruses with a nucleocapsid of helical symmetry. During assembly of the virion, N protein binds to viral RNA, initiating formation of the nucleocapsid. The nucleocapsid is involved in not only viral genome replication, but also in the modulation of various cell signaling pathways. This, coupled with its strong immunogenicity, make the nucleocapsid an important diagnostic tool. The protrusions on spike (S) glycoprotein of coronaviruses will only bind to certain receptors on the host cell and are essential in the binding of the virus to the host cell at the initiation of infection. For example, it has been found that SARS-CoV-2 (COVID-19 coronavirus, 2019-nCoV) infects human respiratory epithelial cells through interaction with the human ACE2 receptor. The spike protein is a large type I transmembrane protein containing two subunits, S1 and S2. The receptor binding domain (RBD),contained within S1, recognizes the cell surface receptor while the S2 region contains basic elements needed for membrane fusion. Also initiated by interactions with the S protein are various T cell responses, neutralizing antibodies, and various other elements of protective immunity. Because of the S protein's importance in infection, it is a target for vaccine development. Targeted regions within the coronavirus on this panel are important in monitoring both the immune response and progression and presence of infection.

Data Analysis

Upon completion of the run by ZIVA, the images for each array are automatically analyzed. This process involves assessing the intensity of each spot assigned to a target analyte (minimum of 3 spots per analyte), calculating the average intensity for each analyte, and normalizing the average intensity to an internal standard on each array to produce the reflective intensity unit (RIU) per analyte.

The negative controls are used to determine the threshold of significance for a positive result. The threshold is based on the 95% confidence interval using a minimum of n=3 negative control replicates and is applied universally to all targets. In addition, the user can use the tag feature in the software for additional controls (negative and/or positive) and export the normalized RIU for customer-specific analysis.

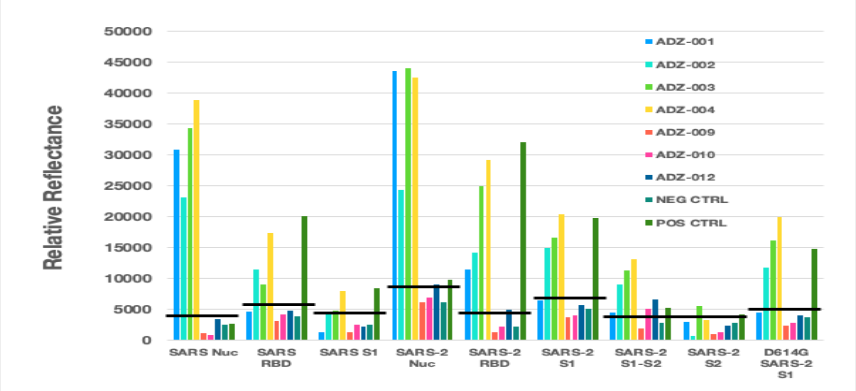


Figure 1. Output of relative reflectance from ZIVA. ADZ-001-ADZ-004 were plasma samples collected > 14 days after positive COVID PCR test ADZ-009-ADZ-012 are plasma samples collected prior to introduction of COVID19

Performance Characteristics

Analytical Sensitivity

The following list of antigens will react with the positive control provided in the assay kit. Each antigen should result in a signal that is a minimum of + 2 SD above the negative control. The analytical sensitivity

*LOQ was determined by estimating the minimal amount of positive control that could be detected on a 95% confidence interval. The LOQ is an estimated range based off 3 independent assay runs.

Antigen	Type of positive control	Estimated LOQ (ng/ml)*
SARS-CoV-2 RBD	Rabbit Polyclonal	95-112
SARS-CoV-2 S1	Rabbit Polyclonal	98-108
SARS-CoV RBD	Rabbit Polyclonal	313-376
SARS-CoV-2 D614G	Rabbit Polyclonal	804-885
MERS-CoV RBD	Rabbit Polyclonal	6,820-8,203
Influenza B Malaysia	Rabbit Polyclonal	53-71
Influenza B Phuket	Rabbit Polyclonal	336-455
Influenza B Massachusetts	Rabbit Polyclonal	1,033-1,356

Analytical Reproducibility

Precision was assessed by analyzing the data from 9 independent assay runs using the positive and negative controls in the kit. Overall, intra-assay and inter-assay precisions were <4.3% and <7.7%, respectively. The precision analysis by antigen target is summarized below:

Target Antigen	Sample	Intra-assay CV%	Inter-assay CV%
SARS-CoV-2 RBD	Positive Ctrl	3.58%	11.13%
	Negative Ctrl	10.02%	11.38%
SARS-CoV-2 S1	Positive Ctrl	3.13%	6.46%
	Negative Ctrl	5.83%	6.77%
SARS-CoV-2 Nuc	Positive Ctrl	2.81%	8.47%
	Negative Ctrl	9.10%	4.78%
SARS-CoV-2 D614G	Positive Ctrl	4.73%	7.61%
	Negative Ctrl	8.66%	9.53%

Sample Performance Results: SARS-CoV-2

Correlation to Viral Status by Polymerase Chain Reaction (PCR)

Samples of known PCR status for the SARS-CoV-2 virus were evaluated using the ZIVA Acute Respiratory Viral Serology Array as well as negative samples (serum/plasma samples collected prior to December 2019). The comparison of performance to samples confirmed positive for SARS-CoV-2 by PCR is shown in Table 1. The results for samples collected prior to December 2019 (presumed negative for SARS-CoV-2) are shown in Table 2.

Table 1: Correlation of ZIVA Array to PCR Positive Samples by Antigen Target

PCR positive, > 14 days after PCR test	Antigen Target	Positive	Negative	Positive Percent Agreement
Serum (N=8)	SARS-CoV-2 S1	7	1	87.5%
	SARS-CoV-2 RBD	7	1*	87.5%
	SARS-CoV-2 Nucleocapsid	7	1	87.5%
	SARS-CoV-2 S1 + S2	7	1	87.5%
	SARS-CoV-2 S2	5	3	62.5%
	SARS-CoV-2 mutant D614G	7	1	87.5%
Plasma (N=7 samples not matched to Serum)	SARS-CoV-2 S1	5	2	71.4%
	SARS-CoV-2 RBD	5	2*	71.4%
	SARS-CoV-2 Nucleocapsid	5	2	71.4%
	SARS-CoV-2 S1 + S2	5	2	71.4%
	SARS-CoV-2 S2	3	4	42.9%
	SARS-CoV-2 mutant D614G	5	2	71.4%

*ELISA testing on PCR positive sample was also negative (see Figure 1)

Table 1: Correlation of ZIVA Array to Negative (CoV-SARS-2) Samples by Antigen Target

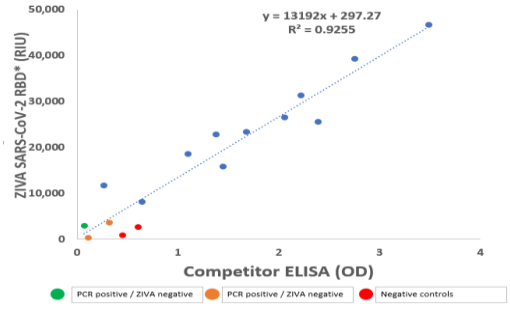
Negative Samples (pre-December 2019)	Antigen Target	Positive	Negative	Negative Percent Agreement
Serum (N=16)	SARS-CoV-2 S1	0	16	100%
	SARS-CoV-2 RBD	0	16	100%
	SARS-CoV-2 Nucleocapsid	0	16	100%
	SARS-CoV-2 S1 + S2	0	16	100%
	SARS-CoV-2 S2	0	16	100%
	SARS-CoV-2 mutant D614G	0	16	100%

*ELISA testing on negative samples were also negative

Correlation to Quantitative ELISA

In addition to performance comparison by PCR status, the performance of the ZIVA SARS-CoV-2 RBD antigen target was correlated to a commercially available ELISA (Figure 1). The results demonstrate excellent correlation (>0.93) of antigen response in RIU (Reflective Intensity Unit) to the quantitative colorimetric ELISA (Optical Density, OD).

Figure 1



Stability

Upon receiving your kit, the box can be stored in 4°C until use. All components are guaranteed for a minimum of 3 months from date of purchase when stored as specified.

Sample Collection and Storage

Both Serum and Plasma have been validated for use in this assay. For additional sample information and suggestions, especially related to freezing and thawing or sample collection, please see the IFU or protocol for the assay.

Ordering information

Item Number	Item Description
MSZ7-ARV-FP3000K	Acute respiratory viral serology array v1.0 27 plex

References

1.Shen S, et al. (2007) Expression, glycosylation, and modification of the spike (S) glycoprotein of SARS CoV. Methods Mol Biol. 379: 127-35. 2.Du L, et al. (2009) The spike protein of SARS-CoV--a target for vaccine and therapeutic development. Nat Rev Microbiol. 7 (3): 226-36. 3.Xiao X, et al. (2004) The SARS-CoV S glycoprotein. Cell Mol Life Sci. 61 (19-20): 2428-30.

2184 Welsch Industrial Court
St. Louis, MO 63146
+1 (314) 925-7800
info@adarzabio.com
www.adarzabio.com