

OVERVIEW

BioServUK SARS-CoV-2 S1 Protein ELISA is a **research use only** sandwich ELISA designed for the quantitative measurement of SARS-CoV-2 S1 Protein in saliva. It uses BioServUK's proprietary Affimer™ technology. The assay employs dual capture Affimer™ reagents binding to the SARS-CoV-2 S1 Receptor Binding Domain with a non-competing biotinylated SARS-CoV-2 S1 detector Affimer™ reagent.

INTENDED USE

BioServUK SARS-CoV-2 S1 ELISA Kit is a highly sensitive and specific immunoassay developed by BioServUK for quantitative detection of the S1 of SARS-CoV-2 in saliva. **This product is intended for research use only. Not for use in diagnostic procedures.**

ASSAY PRINCIPLE

96-well plates are coated with dual capture anti SARS-CoV-2 S1 Affimer™ reagents that capture SARS-CoV-2 S1 protein in the sample. After washing away unbound materials, captured SARS-CoV-2 S1 protein is detected by a non-competing biotinylated SARS-CoV-2 S1 detector Affimer™ reagent and streptavidin conjugated to horse radish peroxidase (HRP). After washing, the chromogenic substrate 3,3',5,5'-tetramethylbenzidine (TMB) is added. Colour reaction is stopped by 0.5M H₂SO₄. The amount of SARS-CoV-2 S1 captured inside the wells is proportional to the colour intensity generated in the coupled oxidation-reduction reaction.

AFFIMER™ PAIR ELISA PROTOCOL – SARS-CoV-2 S1

1. Coat

Prepare a capture reagent mix of 1 µg/mL Capture Affimer (BS -C -CA01) + 1 µg/mL Capture Affimer (BS -C -CA03) in 1x carbonate/bicarbonate buffer (Sigma). Add 100 µL/well into Maxisorp flat-bottomed 96-well plate (Nunc) and incubate for 14-20 h (overnight), 4°C, static.

2. Wash

Wash assay plate with 3 x 300 µL PBS-T (1x PBS + 0.05 % Tween-20). Blot plate dry with paper towel.

NB. All wash steps performed automated on plate washer.

3. Block

Block assay plate with 300 µL/well 1x casein buffer (diluted 1:5 in reagent quality water) and incubate for 2 h, 25°C with gentle agitation.

BSA is not recommended as a blocking buffer component for use with Affimer™ reagents.

4. Standard curve preparation

Prepare SARS-CoV-2 S1 target protein at 2.5 ng/ml in 50% saliva matrix in 1x casein buffer, in triplicate. Transfer to a suitable dilution plate (1 row for each replicate) and carry out 2-fold dilution across the plate down to 0.002 ng/mL in 50% saliva in 1x casein. Perform this step no more than 30 minutes before addition to assay plate with a minimum volume of 120 µL/well.

5. Sample preparation

Prepare QC and test samples in triplicate by diluting saliva samples to 50% in 1x casein buffer. Perform this step no more than 30 minutes before addition to assay plate and keep volumes of target added low to avoid over-dilution of matrix. It is recommended that QC samples are run by spiking the SARS-CoV-2 S1 target at 2 ng/mL, 1 ng/mL and 0.05 ng/mL in 50% saliva to give physiological concentrations of 4, 2 and 0.1 ng/mL.

6. Wash

Wash assay plate with 3 x 300 µL PBS-T (1x PBS + 0.05 % Tween-20). Blot plate dry with paper towel.

7. Sample incubation

Transfer 100 µL target protein titration and samples to blocked assay plate and incubate for 1 h, 25°C with gentle agitation.

8. Wash

Wash assay plate with 3 x 300 µL PBS-T (1x PBS + 0.05 % Tween-20). Blot plate dry with paper towel.

9. Detection – Biotinylated Affimer™ reagent

Prepare 1 µg/mL biotinylated detection Affimer™ reagent (BSV-COV-DET-05) in 1x casein buffer. Add 100 µL/well and incubate for 1 h, 25°C with gentle agitation.

10. Wash

Wash assay plate with 3 x 300 µL PBS-T (1x PBS + 0.05 % Tween-20). Blot plate dry with paper towel.

11. Detection – Streptavidin-HRP

Prepare Streptavidin Poly-HRP reagent at 1:10000 in 1x casein buffer. Add 100 µL/well and incubate for 1 h, 25°C with gentle agitation.

12. Wash

Wash assay plate with 3 x 300 µl PBS-T (1x PBS + 0.05 % Tween-20). Blot plate dry with paper towel.

13. Substrate

Add 100 µL/well room temperature TMB substrate (Surmodics) and incubate at room temperature for 10 m, static. Stop reaction by adding 50 µL/well 0.5M sulphuric acid (Fisher).

14. Analysis

Analyse by measuring absorbance at 450-630 nm using a plate reader. Four-parameter (4PL) non-linear regression analysis used for curve fitting.

RECOMMENDED STORAGE CONDITIONS

It is recommended that the supplied Affimer reagents are stored at 2-8°C

REAGENTS SUPPLIED

- 2 x Capture Affimer™ reagents (BSV-COV-CA01 and BSV-COV-CA03)
- 1 x Detection biotinylated Affimer™ reagent (BSV-COV-DET-05)

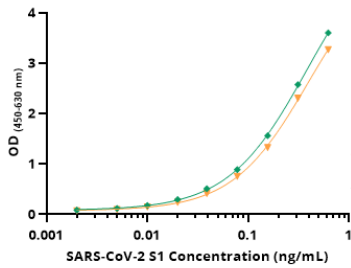
REAGENTS (REQUIRED BUT NOT SUPPLIED)

Reagent	Supplier	Cat #
Maxisorp Plate	Nunc	442404
Carbonate-Bicarbonate Capsules	Sigma	C3041
10x PBS	Gibco	70011-036
Tween-20	Sigma	P9416
PBS Casein ELISA Reagent (5x)	Abcam	ab171532
Streptavidin Poly-HRP	Pierce	21140
TMB Super Sensitive	Surmodics	TMBS-1000-01
Sulphuric Acid Solution 0.5M (1N)	Fisher	J/8430/15

REAGENTS (OPTIONAL)

Reagent	Supplier	Cat #
Normal Saliva - Pooled Human Donors	Lee Bioscience	991-05-P
SARS-CoV-2 S1	Acro Biosystems	S1N-C52H4

TYPICAL PERFORMANCE



		Buffer	50% saliva
		Calibration range	ULOQ
	LLOQ	5 pg/mL	20 pg/mL *
Inter-assay calibration standard metrics	% CV	0.3 - 9.3	0.4 - 4.9
	% Recovery	92.2 - 101.9	98.0 - 107.3
Intra-assay calibration standard metrics	% CV	0.0 - 16.6	0.3 - 17.0
	% Recovery	82.7 - 110.8	95.3 - 112.3

* Physiological range in undiluted saliva

	50% 1.0 ng/mL* (ULOQ)		200 pg/mL* (HQC)		100 pg/mL* (MQC)		50 pg/mL* (LOC)		30 pg/mL* (LLOQ)	
Run	Observed conc (ng/mL)	% recovery	Observed conc (ng/mL)	% recovery	Observed conc (ng/mL)	% recovery	Observed conc (ng/mL)	% recovery	Observed conc (ng/mL)	% recovery
1	0.515159	103.0	0.100793	100.8	0.05133	102.7	0.022894	91.6		
2	0.589541	117.9	0.097556	97.6	0.046114	92.2	0.022634	90.5	0.019121	127.5
3	0.510237	102.0	0.12009	120.1	0.057617	115.2	0.029441	117.8	0.017982	119.9
4	0.449037	89.8	0.09863	98.6	0.046659	93.3	0.024167	96.7	0.014063	93.8
5	0.439879	88.0	0.104691	104.7	0.049105	98.2	0.024743	99.0	0.016215	108.1
6	0.504631	100.9	0.093652	93.7	0.050478	101.0	0.03461	138.4	0.01754	116.9
Mean	0.50		0.10		0.05		0.03		0.02	
StDev	0.053988		0.009325		0.004166		0.004709		0.001936	
%CV	10.8		9.1		8.3		17.8		11.4	
%recov	100.3		102.6		100.4		105.7		113.2	

- Sensitivity has been demonstrated at 40pg/ml in 50% saliva and 20pg/ml in buffer.

CROSS REACTIVITY

This BioServUK ELISA recognizes SARS-CoV-2 S1 only. The assay was challenged with several coronaviruses with no response observed in any of those tested.

- Dual capture 2-domain Affimer sandwich ELISA is specific for SARS-CoV2 S1 with no interaction with other coronaviruses tested.

