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ELISA Path: Total Antibody (SARS-CoV-2,RBD)

3890-1H-R1-1

ELISA^{BASIC} kit with bridging technology for detection of antibodies (immunoglobulins IgG, IgA, IgM) to the SARS-CoV-2 Receptor Binding Domain (RBD) in solutions, e.g., serum/plasma. The ELISA has been validated with human serum/plasma samples from COVID-19 convalescent individuals and uninfected control individuals.

The kit includes	
Strep-Tactin®XT (0.5 mg/ml)	30 µl
Coating buffer	15 ml
Dilution buffer	60 ml
RBD-bridge mix (lyophilized)	1 vial
Standard reconstitution buffer A8	1 vial
Anti-WASP-HRP (200x)	60 µl
TMB substrate	15 ml
Stop solution	15 ml
Wash buffer concentrate (20x)	120 ml
Uncoated ELISA plate	1 plate (96 wells)
Adhesive plate covers	4

To ensure total recovery of the stated quantity vials and bottles have been overfilled.

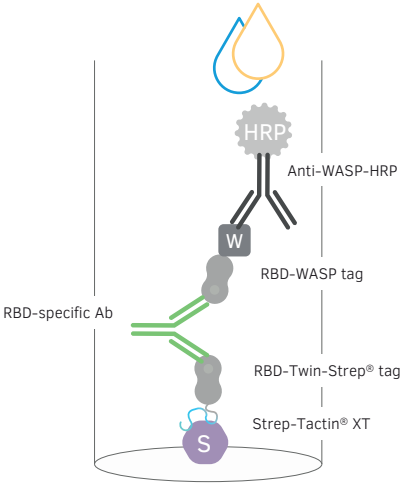
Shipping and storage

Shipped at ambient temperature. All reagents except the RBD-bridge mix should be stored at 2-8 °C upon receipt. The RBD-bridge mix should be stored at -20 °C. Strep-Tactin®XT is supplied in PBS with sodium azide (0.02%). Buffers contain Kathon CG (0.002%). Expiry date indicates how long unopened products, stored according to instructions are recommended for use.

General and Performance

Assay principle

The ELISA plate is coated (overnight) with Strep-Tactin®XT. The sample and the RBD-bridge mix are added after blocking. The RBD-bridge mix contains two components, recombinant RBD with a Twin-Strep-tag®, and recombinant RBD with a WASP peptide tag. Antibodies with specificity to RBD will bind RBD-Twin-Strep-tag® with one binding site and RBD-WASP tag with the other, creating a bridge.
The Twin-Strep-tag will bind to the coated Strep-Tactin®XT, allowing the whole bridge-complex to be captured. The captured complex is detected by addition of anti-WASP-HRP. Addition of TMB substrate will result in a colored substrate product. The reaction is stopped with sulfuric acid and optical density determined using an ELISA plate reader.



Specificity

Specificity – Negative Percent Agreement (NPA) 100% (51/51)

The ELISA[BASIC] assay is intended for detection of immunoglobulins to SARS-CoV-2 RBD in solutions. RBD shows low homology with other coronaviruses that cause common cold in humans, and the risk for cross-reactivity is therefore minimized.

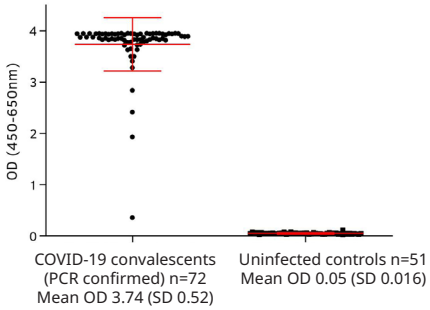
Sensitivity

Sensitivity – Positive Percent Agreement (PPA) 100% (72/72).

Performance data

We recommend to set the cut off OD value as the mean of uninfected controls + 6 SD. The cut off for the data shown was set to OD 0.151

The ELISA is species independent. Analysis of serum/plasma samples from uninfected mouse, cynomolgus and rhesus macaque, rat, cat, cow, dog, rabbit, and pig, all resulted in OD values below cut off.



Preparations

Wash buffer

Add 50 ml Wash buffer concentrate to 950 ml distilled or deionized water (sufficient for all washing steps of 1 plate). If crystals have formed in the 20x concentrate, bring to room temperature and mix gently to dissolve.

Strep-Tactin® XT

Dilute the Strep-Tactin®XT to 1 µg/ml by adding 24 µl Strep-Tactin®XT to 12 ml Coating buffer. Prepare within 15 minutes of use.

Plasma/serum samples

Dilute serum/plasma samples 2x in Dilution buffer within 30 minutes of use.

RBD-bridge mix

Add 500 µl Standard reconstitution buffer to the lyophilized RBD-bridge mix, allow it to dissolve for 5 minutes and mix thoroughly. Use immediately or store in aliquots at -20 °C. For the assay, dilute the RBD-bridge mix 12x in Dilution buffer within 30 minutes of use.

Anti-WASP-HRP

Dilute the anti-WASP-HRP 200x by adding 60 µl anti-WASP-HRP to 12 ml Dilution buffer. Prepare within 15 minutes of use.

Protocol

Day 1

1. Add 100 µl/well of the diluted Strep-Tactin®XT to coat the plate. Incubate overnight at 4-8 °C.

Day 2

2. Discard the coating solution and add 200 µl/well of Dilution buffer to block the plate. Incubate for 1 hour at room temperature.
3. Wash the plate 5 times with Wash buffer (300 µl/well). After the final wash, invert and tap the plate firmly against absorbent paper. Immediately proceed to the next step.
4. Add 50 µl/well of the diluted serum/plasma samples to the plate. Afterwards add 50 µl/well of the diluted RBD bridge mix. Immediately place the plate on an orbital plate shaker (approximately 400 rpm) and incubate for 2 hours at room temperature.
5. Wash the plate as in step 3.
6. Add 100 µl/well of the diluted anti-WASP-HRP. Incubate for 1 hour at room temperature.
7. Wash the plate as in step 3 but **10 times** instead of 5. **(Important!)**
8. Add TMB substrate (100 µl/well) and incubate at room temperature for 15 minutes (protected from direct light).
9. Add Stop solution (100 µl/well) to stop the color development.
10. Measure absorbance at 450 nm (within 15 minutes). If possible, use a reader capable of subtracting a reference wavelength of between 570 and 650 nm.

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The Strep-Tactin® XT Protein contained in this product is manufactured by IBA GmbH and is provided for use in research and commercial market. Use in the commercial market is restricted to companies which own a license for the commercial use of the Twin-Strep-tag. Information about licenses for commercial use of the Twin-Strep-tag is available from IBA GmbH, Rudolf-Wissell-Str. 28, D-37079 Göttingen, Germany.

Updated on 2022/11/17

Developed and manufactured by MABTECH AB, Sweden, whose quality management system complies with the standards ISO 9001:2015 & ISO 13485:2016.

The products are for research use only.

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