SARS-CoV-2 antigen-coated beads

REFERENCE	SIZE	DESCRIPTION
IMS0510	96 tests	Single Receptor-binding domain of S-glycoprotein (RBD) coated beads
IMS0511	96 tests	Single stable trimer of the spicule glycoprotein (S) coated beads
IMS0512	96 tests	Single Nucleocapsid protein (N) coated beads
IMS0513	96 tests	Single Main virus protease or 3C-typeprotese (3CLpro,Mpro) coated beads

1. INTRODUCTION

SARS-CoV-2 is a positive-sense, single-stranded RNA virus that has similarities in genome organisation and expression to SARS-CoV, as well as to other human respiratory coronaviruses (NL63, 229E, OC43 and HKU1) and bat coronaviruses, which is its zoonotic reservoirl. From 5' onwards, two thirds of the SARS-CoV-2 genome encodes for two polyproteins, ppla and pplab, called replicase. These polyproteins are in turn cleaved into 16 non-structural proteins, including RNA-dependent RNA polymerase (RdRp), by the action of two viral proteases essential in virus replication: 3C-type protease (3CLpro) and papain-like protease (PLpro). The other third of the virus genome codes for 8 accessory proteins, which are not essential for replication, and 4 structural proteins, protein S (spicule glycoprotein), protein E (envelope), protein M (membrane) and protein N (nucleocapsid)2 Thus, its genome codes for a total of 28 proteins.

In relation to the viral proteins included in this assay, protein S, which plays a key role in receptor recognition and the cell membrane fusion process, is composed of two subunits, S1 and S2. The S1 subunit contains the receptor-binding domain (RBD) that recognizes and binds to the host receptor's angiotensin converting enzyme 2 (ECA2), while the S2 subunit facilitates fusion between the viral envelope and the plasma membrane of its target cell. On the other hand, the SARS-CoV-2 viral membrane is surrounded by a helical nucleocapsid in which the viral genome is encapsulated by the nucleocapsid protein (N), this viral protein is produced at high levels within infected cells, improving the efficiency of viral RNA transcription and is essential for viral replication. Lastly, the main viral protease (Mpro or 3CLpro) also plays a critical role in viral replication, which is why it has been proposed as a target for specific inhibitors of virus replication3,4 and has been found to be a potent immunogen.

2. INTENDED USE

SARS-CoV-2 antigen-coated beads are intended for the capture of SARS-COV-2 specific antigenic antibodies and later detection by flow cytometry.

3. REAGENTS PROVIDED

SARS-CoV-2 antigen-coated beads are intended for the capture of SARS-COV-2 specific antigenic antibodies and later detection by flow cytometry.

REFERENCE	COMPONENTS
IMS0511	Magnetic polystyrene microspheres with a diameter (µm) 5.5 ± 0.2 (CV< 5%), coated with corresponding antigen each. The microspheres are supplied at the following concentration: 1000 beads/test- 10 µl/test - 960ul/vial and in a buffered aqueous solution containing protein stabilizer and 0.09% sodium azide (NaN_3) ad anti-microbial agent.
IMS0512	
IMS0513	
IMS0514	

4. APPROPIATE STORAGE AND HANDLING CONDITIONS

Store in the dark, refrigerated between 2 °C and 8 °C. DO NOT FREEZE. The kit is stable until the expiry date stated on the vial label if kept at 2-8°C. Do not use after the date indicated.

5. REAGENTS NO PROVIDED

- Magnetic Rack; MagneSphere(R) Mag. Sep. Stand 12-hole, 12x75mm (PROMEGA, Ref Z5343).
- 12x75mm Polystyrene Round Bottom Tubes (cytometer tubes).
- Controls.
- Detection Antibody.
- Wash buffer.
- Sample diluent buffer.

6. EVIDENCE OF DETERIORIATION

Reagents should not be used if any evidence of deterioration is observed. For more information, please contact our technical service: tech@immunostep.com.

7. RECOMENDATIONS AND WARNINGS

a. Avoid microbial contamination of the reagent.
b. Microspheres should be protected from prolonged exposure to light throughout this procedure.
c. Do not use after the expiry date indicated on the vial.
d. Wear personal protective equipment for sample handling. Wash hands properly after handling samples. All procedures should be carried out in accordance with approved safety standards.

8. WARRANTY

Warranted only to conform to the quantity and contents stated on the label or in the product labelling at the time of delivery to the customer. Immunostep disclaims hereby other warranties. Immunostep's sole liability is limited to either the replacement of the products or refund of the purchase price.

9. SAMPLING COLLECTION

Collection of samples (serum, EDTA plasma, heparin or citrate) should be done in suitable collection tubes, using the appropriate anticoagulant. Samples should remain at room temperature for no more than 8 hours. If the assay is to be performed beyond 8 hours, samples should be refrigerated at +2 to +8°C. If the assay is not to be completed within 48 hours of sample extraction, then samples should be stored frozen at - 20°C or below, avoiding unnecessary freeze-thaw cycles. Samples must be properly inactivated. An inactivation protocol may be to hold samples at 56°C for 30 minutes before use.

10. SAMPLING PREPARATION

Samples are diluted 1:20 in sample diluent buffer (IX) (not included- Ref: IMS0514) and mixed with a vortex agitator. Diluted samples should be assayed within 8 hours.

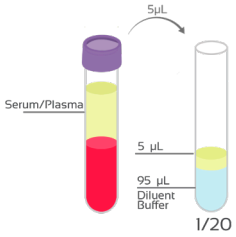

Figure 1: graphical representation of a 1:20 sample preparation. It is recommended to prepare two replicates per sample. It is advisable to work with sample volumes of at least 5 µL to minimize pipetting errors.

11. PROTOCOL

1. **Preparation of the microspheres:** Resuspend each of the magnetic bead vials capped with the viral antigens by vortexing for approximately 20 seconds. Add 10 µL of each of the four antigen-coated beads (RBD, S, N and Mpro) to the tubes listed above. Then, add 10 µL of the sample (See sampling preparation) to the corresponding tube. Shake with a vortex for about 20 seconds.
2. **Incubation of the sample:** Incubate for 60 minutes at room temperature, in the dark and shaking, either tube or plate. When the process is microplate, cover it with the lid.
3. **Washing:** After incubation, wash the sample (antigen-specific antibodies bound to the beads) twice using 0.5 ml (per wash for the tube protocol) or 300 µL (per wash for the microplate protocol) of IX wash buffer for each wash. (Wash buffer not included – Ref. IMS1509). Leave the wash buffer in each tube or well for 30-60 seconds per wash cycle. Subsequently, collect the magnetic beads by placing the tubes or microplate in a magnetic rack or plate respectively and incubate for 5 minutes. Collection of the beads can also be performed by centrifugation at 2500xg for 5 minutes. Remove the supernatant from the tubes or the microplate by manual decanting if using the magnetic plate or rack, or by aspiration if centrifugation is used. Take care not to disturb the beads and make sure to leave a minimum volume of 50 µl and a maximum of 85µl of supernatant in the tube or well.
4. **Incubation of the antibody detection (not included):** take the suggested volume of the primary detection antibody and add it to the cytometer tubes or microplate Wells. Vortex for about 20 seconds and incubate for 30 minutes at room temperature, in the dark and shaking. When the process is microplate, cover the microplate with the lid.
5. **Washing:** After incubation wash 2 times as described above (Step 5).
6. **Acquisition**: Resuspend the sample in 200 µL in PBS and acquire in a flow cytometer or store protected from light for a maximum of 30 min at 2-8°C, until analysis is carried out.

12. AQUISITION AND ANALYSIS IN THE CYTOMETER

A proper bead population selection strategy will allow the removal of doublets and dirt traces, contributing to the correct identification of the 4 bead populations (RBD, S, N and Mpro).

For this purpose, the first step is the selection the bead population on the FSC-H/FSC-A dot plot to remove doublets (A), followed by a selection of the bead population on the SSC-A/FSC-A dot plot to remove dirt traces and reduce background marking (B) is recommended, allowing the correct identification of the 4 bead populations on a dot plot for any of the following channels PerCP/APC, PerCP-Cy5/APC or PerCP-Cy5.5/APC (C).

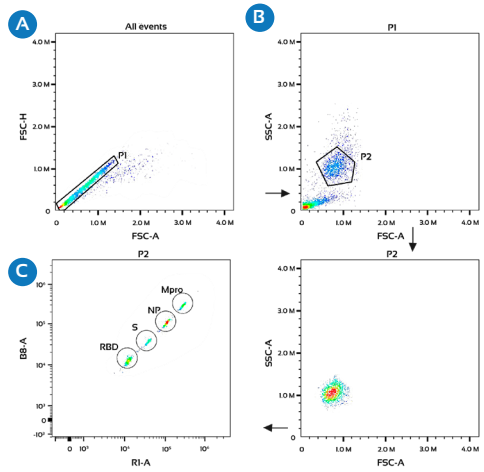

Fig.2: Analysis strategy for bead population selection in the FSC-H/FSC-A A ;SSC/FSC B and PerCP/APC C dotplots.

13. REFERENCES

1. Zhou P, Yang X, Wang X, Hu B, Zhang L, Zhang W et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020;579(7798):270-273.
2. Zhang L, Lin D, Sun X, et al. Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved α-ketoamide inhibitors. Science 2020; 368: 409–412.
3. Martinez-Fleta P, Alfranca A, González-Álvaro I, Casasnovas J, Fernández Soto D, Esteso G et al. SARS-Cov-2 cysteine-like protease (Mpro) is immunogenic and can be detected in serum and saliva of COVID-19-seropositive individuals. 2020;.
4. Cáceres-Martell Y, Fernández-Soto D, Campos-Silva C, García-Cuesta E, Casasnovas J, Navas-Herrera D et al. Bead-assisted SARS-CoV-2 multi-antigen serological test allows effective identification of patients. 2021.

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