SARS-CoV-2 antigen-coated beads

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

abid	Item name
ab326986	SARS-CoV-2 RBD antigen-coated beads
ab326987	SARS-CoV-2 Spike antigen-coated beads
ab326988	SARS-CoV-2 Nucleocapsid antigen-coated beads
ab326989	SARS-CoV-2 3CL-pro(Mpro) antigen-coated beads

For overview, typical data and additional information please visit:

www.abcam.com/ab326986

www.abcam.com/ab326987

www.abcam.com/ab326988

www.abcam.com/ab326989

Storage and Stability: Store in the dark, refrigerated between 2°C and 8°C. DO NOT FREEZE.

Materials Supplied

Item	Quantity	Storage Condition
ab326986 SARS-CoV-2 RBD antigen-coated beads	96 tests	+4°C
ab326987 SARS-CoV-2 Spike antigen-coated beads	96 tests	+4°C
ab326988 SARS-CoV-2 Nucleocapsid antigen-coated beads	96 tests	+4°C
ab326989 SARS-CoV-2 3CL-pro(Mpro) antigen-coated beads	96 tests	+4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

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

Recommendations and Warnings

- Reagents should not be used if any evidence of deterioration is observed.
- Avoid microbial contamination of the reagent.
- Microspheres should be protected from prolonged exposure to light throughout this procedure.
- Do not use after the expiry date indicated on the vial.
- 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.

Sample Collection

Collection of samples (serum, EDTA plasma, heparin or citrate) should be done in suitable collection tubes, using the appropriate anti-coagulant. 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 2-8°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.

Sample Preparation

Samples are diluted 1:20 in sample diluent buffer (1X) (not included) and mixed with a vortex agitator. Diluted samples should be assayed within 8 hours. For example: 5 µL sample with 95 µL sample diluent buffer (1X).

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. 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 1X wash buffer for each wash. (Wash buffer not included). 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 85 µL 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.

Acquisition 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, followed by a selection of the bead population on the SSC-A/FSC-A dot plot to remove dirt traces and reduce background marking 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.

Technical Support

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