ELISpot Path:
SARS-CoV-2 (RBD) Human IgG (ALP)

3850-4APW-R1-1

CONTENTS:

1 pre-coated plate, mAbs MT91/145

For detection of RBD-specific IgG spots:
Antigen: RBD-WASP, lyophilized (1 vial)
Anti-WASP-ALP (60 µl)

For detection of total IgG spots:
Detection mAb:s MT78/145, biotin, 0.5 mg/ml (40 µl)
Streptavidin-ALP (40 µl)

Polyclonal activator: R848, 1 mg/ml, (100 µl)
Lyophilized recombinant human IL-2 (1 µg)
Standard reconstitution buffer A5 (1 ml)

BCIP/NBT-plus substrate (25 ml)

The biotinylated mAbs contain 0.02% sodium azide. Anti-WASP-ALP and SA-ALP are supplied in PBS with 0.002% Kathon CG. Vials have been overfilled to ensure recovery of the speci-fied amount.

STORAGE:

Shipped at ambient temperature. On arrival store RBD-WASP, R848, and IL-2 at -20 °C. Store all other reagents at 4-8 °C. Keep plate at room temp. The expiry date indicates how long unopened products, stored according to instructions, are recommended for use.

Guidelines

PLEASE READ THROUGH BEFORE STARTING THE ASSAY

A Preparation of ELISpot plate (sterile conditions)

1. Remove the plate from the sealed package and wash 4 times with sterile PBS (200 µl/well).
2. Condition the plate with medium (200 µl/well) containing 10% of the same serum as used for the cell suspensions. Incubate for at least 30 minutes at room temperature.

B Incubation of cells in plate (sterile conditions)

1. Remove the medium and add the cell suspension. The number of cells/well needs to be adapted dependent on the type of cells analyzed, e.g., RBD-specific IgG secreting cells or all cells secreting IgG (total IgG). The included R848 and recombinant IL-2 can be used to induce IgG secretion (see Hints and comments).
2. Put the plate in a 37°C humidified incubator with 5% CO_2 and incubate for 18-24 hours. Do not move the plate during this time and take measures to avoid evaporation (e.g. by wrapping the plate in aluminium foil).

C Detection of spots

1. Remove the cells by emptying the plate and wash 5 times with PBS, 200 µl/well.
2. **RBD-specific IgG spots:** Add 500 µl standard reconstitution buffer to the lyophilized RBD-WASP, allow it to dissolve for 5 minutes and mix thoroughly. Dilute the RBD-WASP solution 1:25, e.g. by adding 500 µl RBD-WASP to 12 ml PBS-0.5% FCS. Add 100 µl/well.
   Total IgG spots: Dilute the detection mAbs MT78/145-biotin to 1 µg/ml in PBS-0.5% FCS. Add 100 µl/well.
   Incubate for 2 hours at room temperature.
3. Wash plate as above (step C1).
4. **RBD-specific IgG spots:** Dilute the anti-WASP-ALP (1:200) in PBS-0.5% FCS and add 100 µl/well.
   Total IgG spots: Dilute the Streptavidin-ALP (1:1000) in PBS-0.5% FCS and add 100 µl/well. Incubate for 1 hour at room temperature.
5. Wash plate as above (step C1).
6. Filter the ready-to-use substrate solution (BCIP/NBT-plus) through a 0.45 µm filter and add 100 µl/well. Develop until distinct spots emerge.
7. Stop color development by washing extensively in tap water. If desirable, remove the underdrain (the soft plastic under the plate) and rinse the underside of the membrane.
8. Leave the plate to dry. Inspect and count spots in an ELISpot reader or in a dissection microscope.
9. Store plate in the dark at room temperature.

Hints and Comments

This protocol describes enumeration of B cells secreting IgG antibodies specific for the SARS-CoV-2 Receptor Binding Domain (RBD) and enumeration of all cells secreting IgG (total IgG). The two types of detection are set up in different wells in the plate and require different cell numbers.

Plate washing
Washing of plates can be done using a multi-channel micropipette. In washing steps not requiring sterile conditions (C1-C5), a regular ELISA plate washer can also be used, provided that the washing head is adapted to the ELISpot plates.

Cells
In vivo activated B cells (e.g., during ongoing infection) may be analyzed directly in the ELISpot plate without prior stimulation. Memory B cells may require pre-stimulation *in vitro* with a mixture of R848 (1 µg/ml) and IL-2* (10 ng/ml) in tubes for 3-4 days to secrete detectable amount of antibody. After pre-stimulation, wash the cells extensively to remove secreted antibodies. Freshly prepared and cryopreserved cells may be used. Let the latter rest for at least one hour at 37 °C to allow removal of cell debris. For detection of RBD-specific IgG spots add e.g.,100,000-500,000 cells/well and for total IgG spots e.g., 25,000-50,000 cells/well. Set up samples in triplicates or duplicates.
* Reconstitute IL-2 with 1 ml PBS to obtain 1 µg/ml. Leave for 15 min and then vortex. Use directly or store in aliquots at -20°C.

Serum
The serum should be selected to support cell culture and give low background staining. We recommend the use of fetal calf serum. Alternatively serum-free medium evaluated for cell culture can be used. Human serum should not be used as it contains intrinsic, i.e., IgG which will interfere with the assay.

Assay controls
Preferentially include sample collected pre-SARS-CoV-2. Enumeration of all cells secreting IgG (total IgG) can be including as a control of cell viability.

Buffers
PBS for washing and dilution should be filtered (0.2 µm) for optimal results. Avoid the inclusion of Tween or other detergents in the washing and incubation buffers.

Substrate development
Development is made until distinct spots are visible in positive wells (usually 5-30 minutes). A general darkening of the membrane may occur during development but disappears after drying.

MABTECH

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CERTIFIED ISO 9001 Quality management systems

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