Version 29-12/24

User's Manual

T Cell IL-5 Detection Dot-ELISA Kit

REF **DEIA-JY24022**

Σ **2×96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The T cell Dot-ELISA Kit is one of the most sensitive tests to monitor ex vivo cellular immune responses at single cell level. The assay can accurately detect secreted proteins, such as cytokines, released by e.g. T cells in response to antigen. The cell suspensions, used in the assay, can originate from blood (PBMC), lymphoid, spleen, bone marrow or CNS tissue.

Reagents And Materials Provided

1. Coating antibody* 1 vial 4 °C
2. Biotinylated detection antibody* 1 vial 4 °C
3. Streptavidin-HRP conjugate* 1 vial ≤ -20 °C***
4. AEC Stock solution 1 vial (4 ml) ≤ -20 °C***
5. Substrate buffer (10x) 1 vial (2.5 ml) 4 °C
6. Blocking stock solution (10x) 1 vial (4 ml) 4 °C
7. Dilution buffer R (10x)**** 1 vial (4 ml) 4 °C
8. Tween-20**** 1 vial (5 ml) RT***
9. 96-well plate** with lid 2 plates RT

RT Room temperature (temperature between 20 °C and 26 °C)

* Lyophilized

** PVDF membrane-bottomed Millipore plates

*** Store protected from light

Materials Required But Not Supplied

1. Adhesive cover slips.
2. Tubes and containers/plates to prepare solutions.
3. Sterile distilled water and demineralized water.
4. 70% ethanol.
5. PBS pH 7.4 (home-made). For washing purposes only. Ingredients: $Na_2HPO_4 \cdot 2H_2O$, KH_2PO_4, NaCl and distilled water.
6. Sterile and pyrogen-free liquid PBS pH 7.4 (PBS-I) (Do not use PBS tablets. The filler in the tablets interferes with the coating process.)
7. Cell culture medium: RPMI-1640 supplemented with 2 mM L-Glutamine, 100 units/ml penicillin, 100 µg/ml streptomycin and 10% fetal calf serum.
8. Cell stimuli: antigen of interest and positive control. More information on positive controls at www.ucytech.com/guidelines-stimuli.
9. Laminar flow hood (for sterile conditions), fume hood (for AEC substrate).

10. Pipetting devices.
11. For washing: squirt (wash or squeeze) bottle without sprout. More information on the washing procedure at www.ucytech.com/directions-washing-plates.
12. CO_2 incubator (37 °C, 100% humidity, 5% CO_2).
13. A reflected light microscope or an automated ELISA reader for spot counting.

Storage

Coating and detection antibodies

The vials with lyophilized coating and biotinylated detection antibody can be safely stored at 4 °C until the expiry date (indicated on the vials). After reconstitution, the antibodies are stable at 4 °C for at least 12 months at when kept sterile. However, it is recommended that the reconstituted antibody solutions be divided into small aliquots for single use. These aliquots should be stored at ≤-20 °C (stable for at least two years).

Conjugate

The vial with lyophilized streptavidin-HRP conjugate is stable until the expiry date (indicated on the vial) when stored protected from light at ≤-20 °C. After reconstitution, the reagent is stable at 4°C for at least 2 months when kept sterile and protected from light. However, it is strongly recommended that the solution be divided into small aliquots for single use. These aliquots should be stored protected from light at ≤-20 °C (stable for at least one year).

AEC

The AEC stock solution should be stored at ≤-20 °C and is stable until the expiry date (indicated on the vial)*. Tightly close the vial after use. It is recommended that the solution be divided into small aliquots for single use in polypropylene vials. These aliquots should be stored at ≤-20 °C protected from light (stable for at least one year). * Avoid exposure to light and air: tightly close the vial after use. Avoid contact with polystyrene pipettes and vials.

Substrate buffer

The substrate buffer is stable until the expiry date (indicated on the vial) when stored at 4 °C. Tightly close the vial after use.

Blocking and Dilution buffer

The vials with Blocking stock solution and Dilution buffer can be safely stored at 4 °C until the expiry date (indicated on the vial). After opening, these solutions are stable for at least 6 months when kept sterile.

Tween-20

Tween-20 can safely be stored at RT (protected from light) and is stable until the expiry date (indicated on the vial).

Reagent Preparation

Note: Prepare reagents under sterile conditions (e.g. laminar flow hood).

PBS (for wash buffer)

5. 4 mM $Na_2HPO_4 \cdot 2H_2O$; 1.3 mM KH_2PO_4; 150 mM NaCl in distilled water (adjust to pH 7.4 and filter sterilize [0.2 µm] or autoclave). For 1 ELISA plate: prepare 1 L PBS.

Wash buffer

PBS containing 0.05% Tween-20. For 1 ELISA plate: add 0.5 ml of Tween-20 to 1 L PBS and mix gently but thoroughly. Note: Use PBS only (without Tween-20) as wash buffer in the Human IL-21 T cell ELISA assay.

Blocking buffer (1x)

Dilute Blocking stock solution (10x) in PBS-I.

For 1 ELISA plate: mix 2 ml Blocking stock solution (10x) gently but thoroughly with 18 ml PBS-I.

Dilution buffer (1x)

Dilute Dilution buffer (10x) in PBS-I.

For 1 ELISA plate: mix 2 ml Dilution buffer (10x) gently but thoroughly with 18 ml PBS-I.

Coating antibody

Reconstitute the lyophilized antibody by injecting an appropriate volume (indicated on the vial) of sterile distilled water into the vial. Mix the solution gently for approximately 15 sec and allow it to stand at RT for 5 min. Avoid vigorous shaking.

For 1 ELISA plate (2-plate kit): 100 µl is mixed gently but thoroughly with 5 ml PBS-I.

Detection antibody

Reconstitute the lyophilized antibody by injecting an appropriate volume (indicated on the vial) of sterile distilled water into the vial. Mix the solution gently for approximately 15 sec and allow it to stand at RT for 5 min. Avoid vigorous shaking.

For 1 ELISA plate: 100 µl is mixed gently but thoroughly with 10 ml dilution buffer (1x).

Conjugate

Reconstitute the lyophilized contents by injecting an appropriate volume (indicated on the vial) of sterile distilled water into the vial. Mix the solution gently for approximately 15 sec and allow it to stand at RT for 5 min. Avoid vigorous shaking.

For 1 ELISA plate: 100 µl is mixed gently but thoroughly with 10 ml dilution buffer (1x).

Coloring system

The AEC coloring system consists of two items: a concentrated AEC stock solution* and a concentrated substrate buffer.

For 1 ELISA plate: mix 1 ml Substrate buffer (10x) thoroughly with 4.2 ml 70% ethanol and 4.8 ml demineralized water to reach a final concentration of substrate buffer (1x) in 30% ethanol. Add 660 µl AEC stock solution (toxic: use a fume hood) and mix thoroughly. After mixing the solution should be clear.

This AEC solution should be used within 30 min after preparation.

* AEC stock solution must not come into contact with polystyrene pipettes and vials.

Assay Procedure

Assay controls

Before starting an ELISA experiment, appropriate assay controls need to be chosen, which is mainly dependent on the selected analyte, cell type and experimental set-up.

Positive control: Cells incubated with stimuli (a proven antigen-specific or polyclonal stimulus). For --- Functionality of the cells and whether the assay works well.

Negative control: Cells incubated without stimulus (at the same cell concentration as the experimental antigen of interest). For --- The number of spontaneously secreting cells and false positive results.

Background: No cells but all other reagents. For --- False positive results due to reagents or cell culture media.

Notes:

• A positive and negative control should be tested for each sample on the ELISA plate.

• All assay controls should follow the same procedure and incubation times as the antigen-specific stimulation of the experimental antigen of interest. The only difference between the positive controls and the antigen-specific stimulation/negative control might be a lower final cell concentration per well on the ELISA plate to avoid confluent or poorly defined spots.

• It is recommended to test the samples in triplicate and in serial dilutions in the ELISA procedure. (Since a certain cell number is needed for sufficient stimulation, the assay does not always show linearity in serial dilutions.)

• No more than $3x10^5$ cells/well should be added in the ELISA plate. Higher concentration of cells will cause multiple cell layers, resulting in poor spot formation. For polyclonal stimulation, the recommended cell concentration per well should be reduced to $2x10^2$ – $1x10^5$ cells/well. The volume of the cell preparations in the 96-well ELISA plate is 100 µl/well.

Note: All solutions should be at RT prior to use. Steps 1 till 11 should be performed under sterile conditions. In addition, estimate the time needed to prepare all cell suspensions, which should be ready for step 9, and plan accordingly.

1. Prewet the PVDF membrane of each well of the ELISA plate with 25 µl of 70% ethanol. Incubate for 1 min at RT.
2. Aspirate or firmly shake-out the ethanol. Immediately thereafter wells are rinsed twice with 200 µl PBS-I/well. The plate is subsequently emptied and tapped on tissue paper.
3. Add 50 µl of diluted coating antibody solution into each well of the ELISA plate.
4. Cover the plate with a lid and incubate overnight at 4 °C.
5. Remove coating antibody solution and rinse each well 3x with 200 µl PBS-I. The plate is subsequently emptied with a firm shake-out action.
6. Add 200 µl blocking buffer (1x) into each well.
7. Cover the plate with a lid and incubate for at least 1 h at RT. During this incubation step start preparing the cell sample suspensions*.
8. If the cell suspensions are ready, remove the blocking buffer from the wells with a firm shake-out action (do not wash the wells).
9. Bring the cell suspensions into the wells of the ELISA plate. Add 100 µl/well.
10. Cover ELISA plate with lid and incubate at 37 °C, 5% CO2 and 100% humidity. The incubation time can vary from 24 to 72 h. Specific activation conditions will vary, depending on cell type, protein of interest,

kinetics of protein release and whether a preincubation step was included in the procedure.*

11. Remove the bulk of cells with a firm shake-out action and rinse each well 2x with 200 µl PBS-I. The plate is subsequently emptied.
12. Wash the plate 5x with 250 µl wash buffer/well.*
13. Add 100 µl of diluted detection antibody into each well.
14. Seal the plate with an adhesive cover slip and incubate 2 h at RT (or overnight at 4 °C).
15. Empty plate. Remove and discard the underdrain from the bottom of the plate and wash both sides of the PVDF membrane 5x with wash buffer.
16. Add 100 µl diluted conjugate into each well.
17. Seal the plate with an adhesive cover slip and incubate 1 h at RT protected from light.
18. Empty plate and wash both sides of the PVDF membrane 5x with wash buffer.
19. Add 100 µl freshly prepared AEC solution into each well.
20. Cover plate with lid and incubate for 30 min at RT protected from light.
21. Stop the reaction by emptying the plate and thoroughly rinse both sides of the PVDF membrane with demineralized water.
22. Air-dry the plate at RT (protected from light).
23. Count spots by using a reflected light microscope or an ELISA reader. **Note**: Store the plate at RT at a dry place protected from light to prevent bleaching of spots.

Precautions

This kit is designed for research use only and not for use in diagnostic or therapeutic procedures.