

# Human apoE ELISpot<sup>PLUS</sup> kit

Product Code: 3712-4APW-10

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## CONTENTS:

**10 pre-coated plates, mAb E276**

**Vial 1 (yellow top)**

Biotinylated detection mAb E887 (80 µl)

Concentration 0.5 mg/ml

**Vial 2 (white top)**

Streptavidin-ALP (150 µl)

**BCIP/NBT-plus substrate (120 ml)**

The detection antibody is supplied in sterile filtered (0.2µm) PBS with 0.02% sodium azide. Streptavidin-ALP is supplied in 0.1 M Tris buffer with 0.002% Kathon CG. Vials have been overfilled to ensure recovery of the specified amount.

## STORAGE:

Shipped at ambient temperature. On arrival all reagents should be stored refrigerated at 4-8 °C. Plates should be kept at room temperature. The expiry date indicates how long unopened products, stored according to instructions, are recommended for use.

# Guidelines for Human apoE ELISpot<sup>PLUS</sup>

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 stimuli followed by the cell suspension. Alternatively cells and stimuli can be mixed before addition to the plate.
2. Put the plate in a 37°C humidified incubator with 5% CO<sub>2</sub> and incubate for 18-48 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. Dilute the detection antibody (E887-biotin) to 0.25 µg/ml in PBS containing 0.5% fetal calf serum (PBS-0.5% FCS). Add 100 µl/well and incubate for 2 hours at room temperature.
3. Wash plate as above (step C1).
4. 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

These suggestions are based on the detection of apolipoprotein E secretion using PBMC. If using clones, mixtures of separated cell fractions etc., other protocols may have to be considered.

## **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**

Both freshly prepared and cryopreserved cells may be used in the assay. However it is recommended that the latter are rested for at least one hour to allow removal of cell debris before addition to the plate. As a guideline 100,000-300,000 cells per well can be used. If using purified cell fractions, e.g. monocytes, the cell numbers may have to be reduced to avoid confluent spot formation. It is recommended to include triplicates or duplicates of cell samples. Protocols with other incubation times have to be established by the user.

## **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.

## **Assay controls**

Stimulation with TGF- $\beta$  (1-10 ng/ml) can be used as a positive control for cell viability and functionality of the test system. Stimuli such as LPS down-regulates production of apoE.

## **Detection antibody**

To reduce unspecific background it is recommended to filter (0.2  $\mu$ m) the working dilution of detection mAb.

## **Buffers**

PBS for washing and dilution should be filtered (0.2  $\mu$ m) 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 10-30 minutes). A general darkening of the membrane may occur during development but disappears after drying.

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