ELISpot Plus:
Bovine IL-8 (HRP)

Product Code: 3114-4HPW-2

CONTENTS:

2 pre-coated plates, mAbs MT8H6/8F19

Vial 1 (blue top)
Biotinylated detection mAb 26E5 (40 µl)
Concentration 0.5 mg/ml

Vial 2 (white top)
Streptavidin-HRP (40 µl)

TMB substrate (25 ml)

The detection antibody is supplied in sterile filtered (0.2µm) PBS with 0.02% sodium azide. Streptavidin-HRP is supplied in PBS 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 ELISpot Plus: Bovine IL-8 (HRP)

Please read through before starting the assay

A Preparation of ELISpot plate (sterile conditions)

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)

2. Put the plate in a 37°C humidified incubator with 5% CO_2 and incubate for 12-48 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

2. Dilute the detection antibody (26E5-biotin) to 0.5 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-HRP (1:1000) in PBS-0.5% FCS and add 100 µl/well. Incubate for 1 hour at room temperature.
   Please note that sodium azide used in buffers will inhibit HRP activity.
5. Wash plate as above (step C1).
6. Add 100 µl/well of the ready-to-use TMB substrate solution and develop until distinct spots emerge.
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 immune responses 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. Cell numbers need to be selected based on cell type and stimuli used. For LPS-stimulated bovine PBMC, around 1000-5000 cells/well is recommended.

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
The number of cells responding to stimulation is often compared to the number of cells spontaneously producing cytokine which is determined by incubating the same number of cells in the absence of stimuli. LPS stimulation can be used as a control for cell viability and functionality of the assay.

Detection antibody
To reduce unspecific background it is recommended to filter (0.2 μm) the working dilution of detection mAb.

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. Preferably use deionized water to stop the plates since some ions may cause fading of TMB spots.

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SP CERTIFIED ISO 13485 Medical devices Quality management systems RI. SE

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