

# ELISpot Plus: Bovine IL-8 (ALP)

Product Code: 3114-4APW-10

---

## **CONTENTS:**

**10 pre-coated plates, mAbs MT8H6/8F19**

**Vial 1 (blue top)**

Biotinylated detection mAb 26E5 (150  $\mu$ l)

Concentration 0.5 mg/ml

**Vial 2 (white top)**

Streptavidin-ALP (150  $\mu$ l)

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

The detection antibody is supplied in sterile filtered (0.2 $\mu$ m) 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 ELISpot Plus: Bovine IL-8 (ALP)

Please read through before starting the assay

## **A Preparation of ELISpot plate (sterile conditions)**

2. Condition the plate with medium (200  $\mu$ l/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<sub>2</sub> 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  $\mu$ g/ml in PBS containing 0.5% fetal calf serum (PBS-0.5% FCS). Add 100  $\mu$ l/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  $\mu$ l/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  $\mu$ m filter and add 100  $\mu$ l/well. 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  $\mu\text{m}$ ) the working dilution of detection mAb.

## **Buffers**

PBS for washing and dilution should be filtered (0.2  $\mu\text{m}$ ) 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.

Developed and manufactured by MABTECH AB, Sweden, whose quality management system complies with the standards ISO 9001:2015 & ISO 13485:2016.



**The products are for research use only.**

MABTECH shall not be liable for the use or handling of the product or for consequential, special, indirect or incidental damages there from.

Mabtech AB (Head Office)  
Sweden  
Tel: +46 8 716 27 00  
mabtech@mabtech.com

Mabtech, Inc.  
USA  
Tel: +1 513 871-4500  
mabtech.usa@mabtech.com