References:

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ELISpot for Human Perforin

Product Code: 3465-2AW-Plus

CONTENTS:

- Vial 1 (yellow top)
  Monoclonal antibodies Pf-80/164 (1.2 ml)
  Concentration: 1 mg/ml
- Vial 2 (blue top)
  Biotinylated monoclonal antibody Pf-344 (50 µl)
  Concentration: 1 mg/ml
- Vial 3 (white top)
  Streptavidin-Alkaline Phosphatase (50 µl)
- ELISpot plates (4 PVDF plates)
- BCIP/NBT-plus substrate (2 x 25 ml)

STORAGE:
All reagents should be stored refrigerated at 4-8°C. Plates should be kept at room temperature. Antibodies are supplied in sterile filtered (0.2 µm) PBS with 0.02% sodium azide. Streptavidin-ALP is supplied in 0.1 M Tris buffer with 0.15% Kathon CG®.

To ensure total recovery of stated quantity, vials have been overfilled.
Hints and comments
These suggestions are based on the detection of antigen-specific immune responses using human peripheral blood mononuclear cells (PBMC). If using T-cell clones, mixtures of separated cell fractions etc., other protocols may have to be considered.

Perforin system
Carefully follow the recommended protocol to achieve optimal and reliable results. The spots from perforin secreting cells will be small and distinct and are hard to detect without microscope. The perforin spots normally demands longer substrate development time than e.g. human IFN-γ spots.

Important
Important in all steps: Avoid getting liquid on the backside of the membrane or in the underdrain as this may cause leakage due to capillary drainage.

Plates
Several types of plates can be used in the ELISPOT. However, for optimal results we recommend the use of PVDF-based membrane plates type ELIIP (MAIPSWU10 from Millipore Corp). To obtain maximal antibody binding capacity these plates need to first be activated by a brief treatment with ethanol or methanol. It is essential that the membrane is not allowed to dry after the activation. If this occurs the activation step (A2-3) needs to be repeated before adding the coating antibody.

Plate washing
Always remove the ELIIP plate from the plate tray before manually emptying the plate. 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 with good results. Triplicates or duplicates of 250,000 cells per well are often used to assess antigen-specific responses. For polyclonal activators, the cell number may have to be reduced in order to avoid confluent spot formation. A serum that is good for cell culture and gives low background staining should be chosen; fetal calf serum is normally recommended.

Cell incubation time
Antigen-specific stimulation of cells can result in detectable spots after 18 hours of incubation. If desired, the incubation time can be extended up to 48 hours. Protocols with shorter incubation times have to be developed and evaluated by the user.

Assay controls
The number of cells responding to antigen stimulation is often compared to the number of cells spontaneously producing the cytokine. Spontaneous production is determined by incubating the same number of cells in the absence of antigen. The spontaneous production may be higher for perforin than for the human IFN-γ system. A polyclonal activator such as phytohemagglutinin (1-10 µg/ml) is often included as a control for cell viability and functionality of the test system.

Buffers
PBS for washing and dilution should be filtered (0.2 µm) for optimal results. Although possible to use, we do not recommend the inclusion of Tween or other detergents in the washing and incubation buffers.

Substrate development
Development is made until distinct spots are seen in positive wells (usually 20-50 minutes). A general darkening of the membrane may occur during development but disappears after drying.

For further questions on the kit or protocol, please contact Mabtech.