Datasheet & Protocol

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ELISpot Flex: Rat IFN-γ (HRP)

3220-2H

The kit is intended for enumeration of cells secreting rat IFN-γ using the ELISpot assay.

Components	Concentration	Amount
Capture mAb rIFNγ-I	1 mg/ml	600 µl
Detection mAb rIFNγ-II, biotinylated	1 mg/ml	50 µl
Streptavidin-HRP	-	500 µl

To ensure total recovery of the stated quantity, vials have been overfilled.

Specificity

The kit is based on a matched pair of mAbs specific for rat IFN-γ.

Shipping & Storage

- Shipped at ambient temperature.
- Store at 4-8 °C upon receipt.
- Antibodies are supplied in sterile filtered PBS with 0.02% sodium azide. Streptavidin-HRP is supplied in PBS with 0.002% Kathon CG.
- The expiry date indicates how long unopened products, stored according to instructions, are recommended for use.

Tips and guidance

Plates

ELISpot plates with a PVDF membrane are recommended. An initial ethanol treatment of the membrane gives optimal binding of capture mAb, ensuring a sensitive assay. It is important that the plate membrane is not allowed to dry after treatment. If drying occurs, repeat protocol steps A2-A3 before adding capture mAb.

Plate washing

Plates can be washed using a multi-channel micropipette. When sterile conditions are not required (protocol steps C1-C5), an ELISA plate washer can be used if the washer head position has been adapted to ELISpot plates. After each wash cycle, empty the plate and tap it gently against absorbent paper.

Serum and cell culture medium

For blocking and cell incubation, we recommend cell culture medium containing 10% fetal calf serum (FCS). The serum should be selected to support cell culture and give low background staining. Homologous serum is not recommended. If required, serum-free medium evaluated for cell culture can be used.

Cells

This assay has been validated with spleen cells. For other cell types, the protocol may need adjustment. Freshly prepared or cryopreserved cells can be used. After thawing and washing of cryopreserved cells, leave diluted in cell culture medium at 37 °C for at least one hour. Remove cell debris and count the cells.

Stimuli and assay controls

An assay setup with triplicate wells is recommended. To measure antigen-specific responses, add 200,000-500,000 cells per well. Fewer cells are typically needed for polyclonal activators (used as positive controls for assay functionality), e.g., ConA (1-10 µg/ml). As negative controls, include wells with unstimulated cells and wells with only medium.

Detection antibody

Diluted detection mAb can be filtered (0.2 µm) to reduce the risk of unspecific background.

Development and analysis

A general darkening of the membrane can occur during development but disappears after drying. We recommend analyzing plates in an ELISpot reader (e.g., Mabtech ASTOR or Mabtech IRIS).

Please note

- Do not allow the plate membrane to dry during the assay
- Do not touch the membrane with pipette tips
- Avoid getting liquid on the underside of the membrane as it may cause leakage

Protocol

A. Coating (sterile conditions)

1. Dilute the capture mAb (rIFNγ-I) to 15 µg/ml in sterile PBS.
2. Add ethanol (EtOH) to PVDF plates as follows:
   - MSIP plates (Millipore): 20 µl 35% EtOH per well for maximum 1 minute.
   - MAIPSWU plates (Millipore): 50 µl 70% EtOH per well for maximum 2 minutes.
3. Wash the plate 5 times with sterile water, 200 µl per well.
4. Add 100 µl per well of the diluted capture mAb. Cover the plate and incubate overnight at 4-8 °C.

B. Cell Incubation (sterile conditions)

1. Wash the plate 5 times with sterile PBS, 200 µl per well.
2. Add 200 µl per well of cell culture medium to block and condition the plate. Incubate for at least 30 minutes at room temperature.
3. Empty the plate and add 50 µl stimuli and controls, followed by 50 µl cell suspension per well. Alternatively, mix stimuli and cells before adding 100 µl per well.
4. Place the plate in a 37 °C humidified incubator with 5% CO_2 for 12-48 hours. Avoid evaporation, e.g., by wrapping the plate in aluminum foil. Do not stack or move plates during incubation.

C. Detection

Use PBS containing 0.5% FCS (PBS-0.5% FCS) for dilution of the detection mAb and Streptavidin-HRP. The PBS should be filtered (0.2 µm) for optimal results. Buffers should **not** *contain sodium azide, as it inhibits HRP activity.*

1. Wash the plate 5 times with PBS, 200 µl per well.
2. Add 100 µl per well of the detection mAb (rIFNγ-II-biotin) diluted to 1 µg/ml in PBS-0.5% FCS. Incubate for 2 hours at room temperature.
3. Wash as in step C1.
4. Add 100 µl per well of Streptavidin-HRP diluted* in PBS-0.5% FCS. Incubate for 1 hour at room temperature.
   *The dilution of Streptavidin-HRP should be adapted to the substrate, e.g., 1:1000 for TMB and 1:100 for AEC.
5. Wash as in step C1.
6. Add 100 µl per well of substrate (e.g., TMB, product code 3651-10) and develop until distinct spots emerge (2-20 minutes).
7. Stop color development by washing thoroughly in deionized water (as ions may cause TMB spots to fade).
8. Leave the plate to dry. The underdrain or tray can be removed for quicker drying. Plates should be completely dry before analysis. For the option to re-analyze later, store plates protected from light at room temperature.

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