Cat No.EIS25358Sh

Ovine IFNa ELISpot Kit

Intra assay Precision:≤6%CV
Inter assay Precision:≤10%CV
Storage:-20℃; short-term storage at 4℃ is acceptable for up to 2 weeks
Uses: For the quantitative determination of the frequency of cells releasing For the quantitative determination of the frequency of cells releasing Ovine IFNa
Specifications:96T
Production Date: See outer packaging
Shelf Life:12 months when stored at -20℃

Ovine IFNa ELISpot Kits are highly sensitive, microplate-based assays for the detection of cytokine secreting cells.
Ovine IFNa ELISpot Kits complete microplate-based immunoassays designed for the detection and enumeration of single cells secreting cytokines or other antigens. These assays are highly sensitive and can quantitate actively secreting cells even when cell frequencies fall below 1 in 100,000. Unlike other methods that assess antigen-specific immune cell responses, this assay does not require prior in vitro expansion of cells and is suitable for high-throughput analysis. Ovine IFNa ELISpot Kits are complete ready-to-run assays and require no further development or refinement.
Ovine IFNa ELISpot Kits employ antibody-coated, membrane-backed microplates to identify secreting cells. The immobilized capture antibodies bind to cytokines that have been secreted in their vicinity, and enzyme conjugates are used to visualize the secreted proteins. Colored precipitates form at the sites of cytokine localization and appear as spots, with each individual spot representing an individual cytokine-secreting cell.

Other supplies required

1. Dissection microscope or an ELISpot reader.
2. Pipettes and pipette tips.
3. Deionized water.
4. Squirt bottle, manifold dispenser, or automated microplate washer.
5. 500 mL graduated cylinder.
6. 37 °C CO2 incubator.
7. Sterile culture media.

Kit Components

Name	96 Tests	48 Tests	Storage
Ovine IFNa ELISpot Kit Pre-coated plate	8×12	8×6	4/-20℃
Ovine IFNa ELISpot Kit Positive Control,15ng	2 vial	1 vial	4/-20℃
Biotinylated Ovine IFNa ELISpot Kit antibody (1:100)	1vial	1 vial	4/-20℃
Enzyme conjugate(1:100)	1vial	1 vial	4/-20℃
Ovine IFNa ELISpot Kit Antibody diluent	1vial	1 vial	4/-20℃
Enzyme diluent	1vial	1 vial	4/-20℃

Washing buffer (1:25)	1vial	1 vial	4/-20℃
Color Reagent(BCIP/NBT Substrate)	1vial	1 vial	4/-20℃

Reagent Preparation

Wash Buffer: Dilute the concentrated washing buffer with double-distilled water at a ratio of 1:25. Store the unused portion back in the original kit box.

Ovine IFNa ELISpot Kit Positive Control: Reconstitute the lyophilized Ovine IFNa ELISpot Kit Positive Control with 250 µL of culture medium that is used to incubate cells.

Ovine IFNa ELISpot Kit Detection Antibody working solution: Remove the appropriate volume of Biotinylated Antibody solution based on the number of wells to be assayed, and dilute it with Antibody Diluent at a 1:100 ratio. Mix the solution thoroughly after dilution.Prepare this working solution 30 min in advance, and do not reuse the diluted solution for subsequent assays.

Streptavidin-AP working solution: Remove the appropriate volume of Enzyme Conjugate solution based on the number of wells to be assayed, and dilute it with Enzyme Diluent at a 1:100 ratio. Mix the solution thoroughly after dilution. Prepare this working solution 30 min in advance, and do not reuse the diluted solution for subsequent assays..

Sample Preparation

The types of effector and responder cells used, method of cell separation, mode of stimulation, and length of incubation are to be determined by each investigator.

Assay Procedure

Bring all reagents to room temperature, except the diluted Detection Antibody Mixture,which should remain at 2-8 °C. All samples and controls should be assayed at least in duplicate.

1. Fill all wells in the microplate with 200 µL of sterile culture media and incubate for approximately 20 minutes at room temperature.
2. When cells are ready to be plated, aspirate the culture media from the wells. Immediately add 100 µL of the appropriate cells or controls to each well.
3. Incubate cells in a humidified 37 °C CO2 incubator. Optimal incubation time for each stimulus should be determined by the investigator. Do not disturb the cells during the incubation period.
4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (250-300 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 100 µL of the diluted Detection Antibody working solution into each well, and incubate overnight at 2-8 °C. Alternatively, incubation with detection antibodies can be done for 2 hours at room temperature on a rocking platform.
6. Repeat the wash procedure described in step 4.
7. Add 100 µL of the Streptavidin-AP working solution into each well, and incubate for 2 hours at room temperature.
8. Repeat the wash procedure described in step 4.
9. Add 100 µL of the BCIP/NBT Substrate into each well, and incubate for 1 hour at room temperature. Protect from light.
10. Decant the BCIP/NBT Substrate from the microplate and rinse the microplate with deionized water. Invert

the microplate and tap to remove excess water. Remove the flexible plastic underdrain from the bottom of the microplate, wipe the bottom of the plate thoroughly with paper towels and dry completely either at room temperature (60-90 minutes) or 37 °C (15-30 minutes).

Calculation of Results

The developed microplate can be analyzed by counting spots using either a dissection microscope or an ELISpot reader. Specific spots are round and have a dark center with slightly fuzzy edges. Quantitation of results can be done.

Reproducibility Data

The sample was assayed in seven wells according to the procedure and analyzed with a dissection microscope.

Well	1	2	3	4	5	6	7
Number of Spots Counted	122	141	123	171	168	138	141

Note

1.The reconstituted positive control cannot be stored after preparation; do not refreeze the positive control once it has been reconstituted.

2.Due to shaking or inversion during transportation, centrifugation of the kit tubes or bottles may be necessary to collect all contents at the bottom. Tubes should be vortexed manually or centrifuged at 1000 rpm for 1 min.

3.Concentrated washing buffer may crystallize slightly. Heat the buffer in a water bath to facilitate dissolution during dilution. Crystals must be completely dissolved before the washing buffer is used.

4.The prepared positive control is for single-use only; do not reuse any positive control that has already been assayed. Use the second vial provided if the assay needs to be repeated.

5.Use only the reagents and components provided with this kit. Do not mix batches or lots from other orders of this kit or from different kit types.

6.Ensure all reagents are thoroughly mixed. For reagents added to the microplate, adequate mixing is critical for obtaining accurate assay results.

7.Bring the kit to room temperature before starting the assay.

8.Store unused microplate strips in the original foil bag at 2–8℃ if they will be used within a short period.

9.Do not use kits that have exceeded their expiration date.

10.All samples, washing buffer, and wastes should be treated as biohazardous waste.

11.Sample addition must be performed using a pipette or similar calibrated instrument. Calibrate the instrument before the assay to avoid experimental errors. Add samples to the wells quickly, and control the total sample addition time to less than 5 minutes. A multichannel pipette is recommended to reduce loading time.

12.Regularly verify the calibration of the incubator to ensure a stable incubation temperature of 37℃.

13.For the 48-well ELISpot Kit, all component volumes are 50% of those provided in the 96-well kit.