TECHNICAL MANUAL

Rabbit IL-4 ELISpot Kit

- **SKU CODE:** KESP0188
- **DETECTION PRINCIPLE:** ELISpot
- **RUO:** Research-Use-Only

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Rabbit IL-4 ELISpot Kit

Please read entire manual carefully before starting experiment.

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1. Key Features

Reactivity:

Rabbit

Sample Type:

Cell samples, including fresh or cryopreserved peripheral blood mononuclear cells (PBMCs) and other cultured cell types

Kit Type:

Antibody pair set for the development of ELISpot assays, containing capture antibody, standard control and biotinylated detection antibody for rabbit IL-4. Sufficient for the development of up to two (2) ELISpot assays.

Detection:

Colorimetric

2. Storage & Expiry

Assay Genie ELISpot Kits are shipped on ice packs. Please store this ELISpot Kit and/or components at 2°C to 8°C on receipt. The kit is stable for 12 months from the date of receipt when stored as directed. The date of expiration is on the kit box label.

3. Product Description

The Assay Genie Rabbit IL-4 ELISpot Kit is highly sensitive enzyme-linked immunosorbent "spot" assay designed to quantify the number of individual cells producing rabbit IL-4. Unlike conventional ELISA assays, which measure the total concentration of a protein in a sample, the ELISpot assay quantifies single cells actively secreting the target protein.

In this assay, capture and detection antibodies specific to the analyte are used to bind the molecule as it is released by the cell. Following the addition of a substrate, an enzyme-linked detection system produces a colorimetric precipitate, forming discrete spots on the membrane. Each spot represents a single protein-secreting cell, allowing for sensitive and quantitative assessment of immune responses at the cellular level.

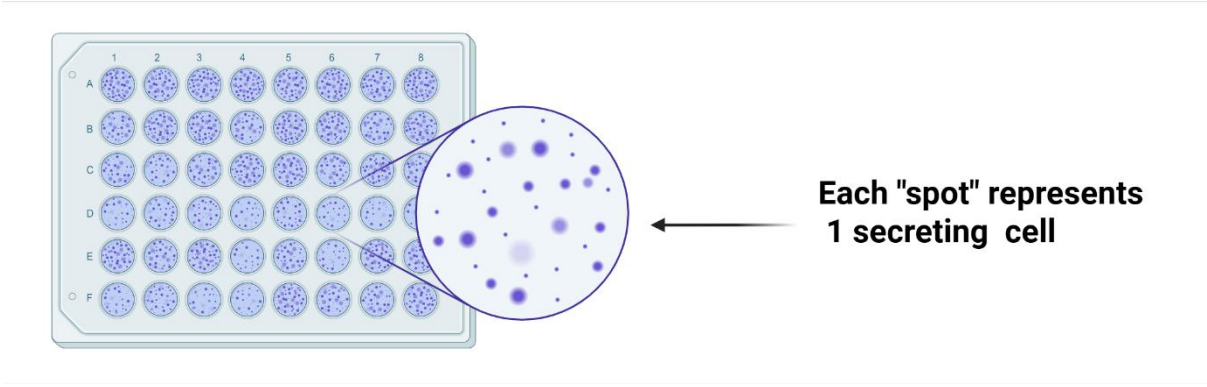


Figure 1: Graphical representation of a 96-well ELISpot Assay results. Each spot that develops in the assay represents a single reactive cell.

4. Kit Contents

No	Component Name	Size
1	Anti-Rabbit IL-4 Polyclonal Antibody (Capture Antibody)	100 µg
2	Recombinant Rabbit IL-4 (Standard Control)	5 µg
3	Biotinylated Anti-Rabbit IL-4 Polyclonal Antibody (Detection Antibody)	50 µg × 2

This kit contains the capture antibody, standard control and detection antibody required for the development of up to two (2) ELISpot assays. All other materials and reagents required are listed in Section 5 and are not supplied. The antibodies have been determined to function in an ELISpot with the standard control provided. Components may also be purchased separately.

5. Materials & Reagents Required but Not Provided

Material / Reagent	Suggested Formulation
96-well ELISpot Plate	96-well, sterile, filter membrane ELISpot plate
Sterile DPBS	0.008 M sodium phosphate, 0.002 M potassium phosphate, 0.14 M sodium chloride, 0.01 M potassium chloride, pH 7.4, sterile filtered
Complete Medium / Blocking Buffer	Sterile medium containing 10% heat-inactivated Fetal Bovine Serum (FBS) or Equine Serum (ES) in the desired cell culture medium, such as RPMI. If required, serum-free medium for cell culture can also be used.
Reagent Diluent	4% BSA in DPBS, 0.2 µm filtered
Wash Buffer	0.05% Tween®-20 in DPBS
Streptavidin-AP	Enzymatic reagent to react with the biotinylated detection antibody
BCIP/NBT Substrate	5-bromo-4-chloro-3-indolylphosphate and nitroblue tetrazolium substrate to react with AP

6. Precautions

1. Store all reagents at the recommended temperature.
2. Do not use reagents if they become visibly contaminated during storage.
3. Reagents should be at room temperature before use.
4. Prepare reagents immediately prior to use. Buffers may be prepared in advance.
5. Vigorous plate washing is essential; however, care should be taken to avoid puncturing the filter membrane ELISpot plate during wash steps. If using an automatic plate washer, confirm and adjust the height of the manifold dispenser prior to use.
6. Use new disposable pipette tips for each transfer to avoid cross-contamination.
7. Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
8. Avoid microbial contamination of reagents and equipment. Automated plate washers can easily become contaminated thereby causing assay variability.
9. Take care not to contaminate the BCIP/NBT Solution. Do NOT return leftover BCIP/NBT Substrate to bottle. If the solution is blue before use, DO NOT USE IT.
10. Individual components may contain preservatives. Wear gloves while performing the assay. Please follow proper disposal procedures.
11. Do not use sodium azide as a preservative for buffers. Sodium azide interferes with substrate reactions.

7. Sample Preparation

Note: *The procedures outlined in this document are provided as general recommendations for sample preparation in ELISpot assays. Due to the variability of biological samples and specific assay requirements, users are advised to optimize protocols based on their own experimental conditions.*

General Considerations

- All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions when handling and disposing of infectious agents.
- Samples should be assayed in triplicate for each experiment.
- Once stimulated cells are added, do not agitate the plates during incubation.
- To avoid edge effects, do not stack plates during incubation.
- If an excessive number of spots is anticipated, prepare serial dilutions of cells and/or stimuli to ensure results remain within the optimal quantitation range.
- If the integrity of the sample is of concern, make a note on the Plate Template and interpret results with caution.

A. Cell Samples Processing

The assay can be optimized for various cell types. See below the protocol for the most common cell samples:

Blood Cells:

- All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions when handling and disposing of infectious agents.
- Fresh Peripheral Blood Mononuclear Cells (PBMCs) obtained by Ficoll-Hypaque density gradient separation can be used in the ELISpot assay.

Cryopreserved Cells

- Cryopreserved PBMCs or other cell types may also be used in the assay.
- After thawing, rest the cells for 1 hour at 37°C, 5% CO_2 before counting and use.
- To reduce clumping during the initial thawing step, add >50 U/mL recombinant nuclease (e.g., Benzonase) to the Complete Medium wash.
- After treatment, resuspend cells in fresh Complete Medium before plating.

B. General Protocol for Sample Handling with ELISpot Assay

The following protocol describes how to use any sample types (including blood cells and cryopreserved cells) with ELISpot Assay:

- Prior to the addition of any stimulant, cells should be washed thoroughly to remove interfering substances such as naturally produced cytokines.
- Use a total sample volume of 100 µL per well.
- During assay optimization, test a broad range of cell concentrations (e.g., $2 \times 10^3 - 2 \times 10^6$ cells/mL) to determine the optimal density for distinct spot formation.
- Prepare cells and stimuli at 2X concentration in Complete Medium. Add 50 µL of stimuli and 50 µL of cells to each well to achieve the desired 1X final concentration.
- Alternatively, cells and stimuli can be pre-mixed at the final concentration and added as 100 µL per well.

For more information about how to process other sample types, please contact our Tech Support Team at techsupport@assaygenie.com.

8. Suggested Controls

Control	Suggested Formulation
Standard Control	Recombinant rabbit IL-4 at 50 ng/mL in Complete Medium.
Blank Control	Complete Medium only.
Positive Control	Cells incubated with a polyclonal activator such as PHA, PMA/Ionomycin, ConA, or other, as desired.
Negative Control	Cells in Complete Medium only.

9. Assay Procedure

Time Requirements

Time Required	Assay Stage
1 overnight incubation	Coated plate preparation
1–2 overnight incubations	Cell stimulation, as desired
2.25+ hours	Assay development

Sterile Conditions Suggested

1. Prepare Capture Antibody Working Solution in DPBS at 4 µg/mL, or as desired.

2. Add 100 µL of Capture Antibody Working Solution to each well.

3. Cover plate and incubate at room temperature (20–25°C) for 12–24 hours.

4. Empty Capture Antibody Working Solution from each well.

5. Add 250 µL of Complete Medium/Blocking Buffer to each well.

6. Cover plate and incubate at room temperature for 1–3 hours.

Tip: *Prepare controls and samples for ELISpot during Blocking Buffer incubation.*

7. Empty Blocking Buffer from each well.

8. Add 100 µL of controls or samples to appropriate wells.

Note: *Run each control or sample in triplicate.*

9. Cover plate and incubate at 37°C, 5% CO_2 for 12–48 hours, as desired.

No Sterile Conditions Required

10. Wash plate SIX times with Wash Buffer.

Note: *Vigorous plate washing is essential; however, care should be taken to avoid puncturing the filter membrane ELISpot plate during wash steps. If using an automatic plate washer, confirm and adjust the height of the manifold dispenser prior to use. Take*

care to avoid microbial contamination of equipment. Automated plate washers can easily become contaminated thereby causing assay variability.

11. Prepare Detection Antibody Working Solution in Reagent Diluent at 4 µg/mL, or as desired.

12. Add 100 µL of Detection Antibody Working Solution to each well.

13. Cover plate and incubate at room temperature for 1 hour.

14. Wash plate SIX times with Wash Buffer as described in step 10.

15. Prepare Streptavidin-AP in Reagent Diluent at the desired working concentration.

16. Add 100 µL of Streptavidin-AP Working Solution to each well.

17. Cover plate and incubate at room temperature for 1 hour.

18. Wash plate SIX times with Wash Buffer as described in step 10.

19. Add 100 µL of BCIP/NBT Substrate Solution to each well.

20. Develop the plate at room temperature for up to 15 minutes.

21. Stop reaction by flooding the plate with ultrapure water. Remove under tray and flood bottom of tray with ultrapure water.

22. Dry plate and evaluate results.

Notes:

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.