

# Rabbit IL-1 $\alpha$ Do-It-Yourself ELISpot



**Catalog Number:** SPOT2512U-004

**Storage:** 2-8°C

**Stability:** 12 months (from date of receipt)

**Country of Origin:** USA

**Description:** Contains Capture Antibody, Standard Control, and Detection Antibody for development of up to two (2) ELISpot assays. Other required materials and reagents are not supplied. The antibodies have been determined to function in an ELISpot with the Standard Control provided. Components may also be purchased separately.

**Included Components:**

| Description                                                | Usage              | Quantity       | Catalog Number |
|------------------------------------------------------------|--------------------|----------------|----------------|
| Anti-Rabbit IL-1 $\alpha$ Polyclonal Antibody              | Capture Antibody   | 100 $\mu$ g    | KP1060U-100    |
| Recombinant Rabbit IL-1 $\alpha$                           | Standard Control   | 5 $\mu$ g      | RP0346U-005    |
| Biotinylated Anti-Rabbit IL-1 $\alpha$ Polyclonal Antibody | Detection Antibody | 50 $\mu$ g x 2 | KPB1061U-050   |

**Additional Materials and Reagents Required:**

| Material/Reagent                    | Suggested Formulation                                                                                                                                                                                        |
|-------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 96-well ELISpot Plate               | 96-well, sterile, filter membrane ELISpot plate<br><i>ELISpot Plate: Catalog # AR2307-001</i>                                                                                                                |
| Sterile DPBS                        | 0.008M sodium phosphate, 0.002M potassium phosphate, 0.14M sodium chloride, 0.01M potassium chloride, pH 7.4, sterile filtered                                                                               |
| Complete Medium/<br>Blocking Buffer | Sterile medium containing 10% heat-inactivated Fetal Bovine Serum (FBS) or Equine Serum (ES) in 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 $\mu$ m filtered                                                                                                                                                                         |
| Wash Buffer                         | 0.05% Tween®-20 in DPBS                                                                                                                                                                                      |
| Streptavidin-AP                     | Enzymatic reagent to react with biotinylated detection antibody<br><i>Streptavidin-AP: Catalog # AR2308-001</i>                                                                                              |
| BCIP/NBT Substrate                  | 5-bromo-4-chloro-3-indolylphosphate and nitroblue tetrazolium substrate to react with AP.<br><i>BCIP/NBT Substrate: Catalog # AR2309-025</i>                                                                 |

**Time Requirements:**

|                          |                             |
|--------------------------|-----------------------------|
| 1 overnight incubation   | Coated plate preparation    |
| 1-2 overnight incubation | Cell stimulation as desired |
| 2.25+ hours              | Assay development           |



## Reagents for Animal Model and Animal Health Research

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# Do-It-Yourself ELISpot

## Suggested ELISpot Controls:

| Control          | Suggested Formulation                                                                               |
|------------------|-----------------------------------------------------------------------------------------------------|
| Standard Control | Recombinant Protein 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.                                                                      |

## ELISpot Protocol: 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<sub>2</sub> for 12-48 hours, as desired.  
DO NOT DISTURB PLATE DURING INCUBATION!

## 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 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.

## Warranty:

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