Signosis
BioSignal Capture

# Rat IL-1α ELISA

**Catalog Number EA-3004** **(For Research Use Only)**

## Introduction

IL-1α and IL-1β are prominent agonists mediating inflammatory and immunomodulatory effects. Both are produced by macrophages, monocytes and dendritic cells. They are important part of the inflammatory response against infection. They increase the expression of adhesion factors on endothelial cells to enable transmigration of leukocytes to sites of infection. IL-1α is a pleiotropic cytokine involved in various immune responses, inflammatory processes, and hematopoiesis. It is produced by many cell types but is only secreted by monocytes and macrophages.

## Principle of the assay

IL-1α ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay utilizes rabbit anti-rat IL-1α for immobilization on the microtiter wells and biotinated rabbit anti-rat IL-1α antibodies along with streptavidin conjugated to horseradish peroxidase (HRP) for detection. The test sample is allowed to react simultaneously with the two antibodies, resulting in the IL-1α molecules being sandwiched between the solid phase and enzyme-linked antibodies. After incubation, the wells are washed to remove unbound-labeled antibodies. A HRP substrate, TMB, is added to result in the development of a blue color. The color development is then stopped with the addition of Stop Solution changing the color to yellow. The concentration of IL-1α is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

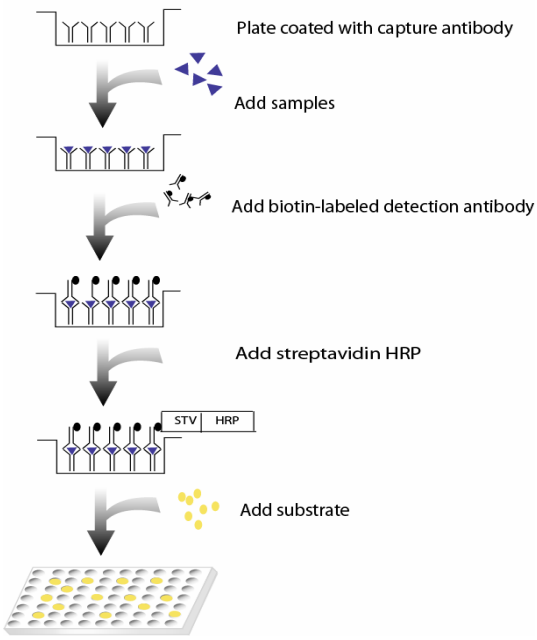


Diagram of ELISA

## Materials provided with the kit

- 96 well microplate coated with rabbit anti-rat IL-1α antibodies (4°C).
- Biotin labeled rabbit anti-rat IL-1α antibodies (-20°C).
- Streptavidin-HRP conjugate (4°C).
- Recombinant Rat IL-1α standard (1000ng/ml) (-20°C).
- 1X Diluent buffer (4°C).
- 5X Assay wash buffer (RT)
- Substrate (4°C).
- Stop Solution (4°C).

## Material required but not provided

- Microplate reader capable of measuring absorbance at 450 nm
- Deionized or distilled water.

## Reagent preparation before starting experiment

- Dilute the 5x Assay wash buffer to 1x buffer
  40ml 5x Assay wash buffer
  160ml ddH2O
- Dilute 500 times of Rat recombinant IL-1α (1000ng/ml) with 1X Diluent buffer to 2000pg/ml and then 2-fold serial dilutions.
- Dilute 400 times of biotin labeled rabbit anti-rat IL-1α antibodies with 1X Diluent buffer before use.
- Dilute 200 times of streptavidin-HRP with 1X Diluent buffer before use.

## Assay procedure

1. Cut the sealing film over the plate and remove it from the desired number of wells. Make sure the rest of wells are well sealed.
2. Add 100 µl of Standard, control, or sample per well and incubate for 1 hour at room temperature with gentle shaking.
3. Aspirate each well and wash by adding 200µl of 1X Assay wash buffer. Repeat the process three times for a total of three washes. Complete removal of liquid at each wash. After the last wash, remove any remaining liquid by inverting the plate against clean paper towels.
4. Add 100 µl of diluted biotin-labeled rabbit anti-rat IL-1α antibodies to each well and incubate for 1 hour at room temperature with gentle shaking.
5. Repeat the aspiration/wash as in step 3.
6. Add 100 µl of diluted streptavidin-HRP conjugate to each well and incubate for 45 min at room temperature with gentle shaking.
7. Repeat the aspiration/wash as in step 3.
8. Add 100µl of substrate to each well and incubate for 5-30 minutes.
9. Add 50µl of Stop solution to each well. The color in the wells should change from blue to yellow.
10. Determine the optical density of each well with a microplate reader at 450 nm within 30 minutes.

## Example of standard curve