# Rat IL-6 One-Step ELISA Kit Datasheet

Please read it entirely before use

**Catalogue Number:** SE70002

**Size:** 96T

**Sensitivity:** 11.5 pg/mL

**Range:** 7.8-500 pg/mL

**Usage:** For the quantitative detection of rat IL-6 concentrations in serum, plasma and cell culture supernatant.

**This product is for research use only and not for use in human or animal therapeutic or diagnostic.**



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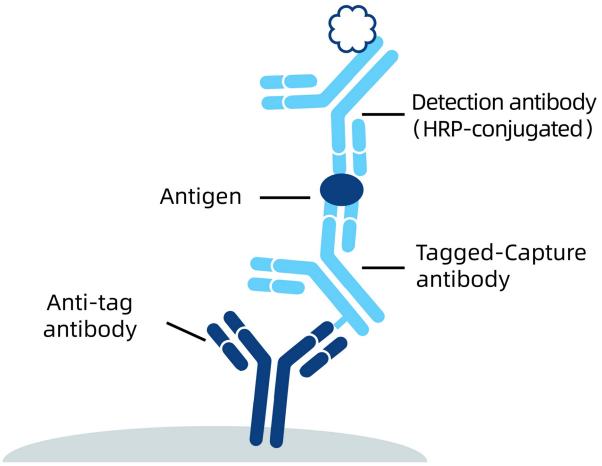
## 1. Background

Interleukin-6 (IL-6) is an interleukin that acts as both a pro-inflammatory and anti-inflammatory cytokine. IL-6 protein is secreted by a variety of cell types including T cells and macrophages as phosphorylated and variably glycosylated molecule. IL-6 plays an essential role in the final differentiation of B-cells into Ig-secreting cells involved in lymphocyte and monocyte differentiation. It induces myeloma and plasmacytoma growth and induces nerve cells differentiation. IL-6 is also considered a myokine, a cytokine produced from muscle, and is elevated in response to muscle contraction. IL-6 has been shown to interact with interleukin-6 receptor and glycoprotein 130. Additionally, IL-6 is involved in hematopoiesis, bone metabolism, and cancer progression, and has been defined an essential role in directing transition from innate to acquired immunity.

## 2. Principle




An anti-tag antibody is pre-coated onto the bottom of wells. After adding antigen or samples, Tagged-Capture antibody and HRP-conjugated detection antibody, a sandwich complex is formed in the solution. TMB acts as a HRP substrate, and the solution color will change from colorless to blue. A stop solution containing sulfuric acid turns the solution yellow. The color intensity is proportional to the quantity of bound protein, which is measurable at 450 nm with the correction wavelength set at 630 nm.

## 3. Required Materials

3.1 A microplate reader capable of measuring absorbance at 450 nm with the correction wavelength set at 630 nm.

3.2 Calibrated, adjustable precision pipettes and disposable plastic tips. A manifold multi-channel pipette is recommended for large assays.

3.3 Plate washer: automated or manual.

3.4 Absorbent paper towels.

3.5 Glass or plastic tubes to prepare standard and sample dilutions.

3.6 Beakers and graduated cylinders.

3.7 Log-log or semi-log graph paper or computer and software for ELISA data analysis. A four-parameter logistic (4-PL) curve-fit is recommended. Proteintech data analysis website, https: //www.ptgcn.com/products/elisa-kits/.

3.8 Microplate thermostatic shaker.



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## 4. Kit Components and Storage

| | | |
|---|---|---|
| **Microplate** - 96 well microplate precoated an anti-tag antibody(8 well × 12 strips) | 1 plate | **Unopened Kit:** Store at 2-8°C for 6 months or -20°C for 12 months. **Opened Kit:** All reagents stored at 2-8°C for 7 days. **Please use a new standard for each assay.** |
| **Protein standard** - 1000 pg/bottle; lyophilized | 2 bottles | |
| **Capture antibody (100X)** - 60 µL/vial* | 1 vial | |
| **Detection antibody, HRP-conjugated (100X)** - 60 µL/vial* | 1 vial | |
| **Sample Diluent PT 3** - 30 mL/bottle | 1 bottle | |
| **Detection Diluent** - 15 mL/bottle | 1 bottle | |
| **Wash Buffer Concentrate (20×)** - 30 mL/bottle | 1 bottle | |
| **Tetramethylbenzidine Substrate (TMB)** - 12 mL/bottle | 1 bottle | |
| **Stop Solution** - 12 mL/bottle | 1 bottle | |
| **Plate Cover Seals** | 4 pieces | |

* Centrifugation immediately before use

## 5. Safety Notes

5.1 Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

5.2 Do not use the kit after the expiration date.

5.3 Do not mix or substitute reagents or materials from other kit lots or other sources.

5.4 Be sure to wear protective equipment such as gloves, masks and goggles during the experiment.

5.5 When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer to improve assay precision

## 6. Sample Collection and Storage

6.1 Serum: Allow blood samples to clot for 30 minutes, followed by centrifugation for 15 minutes at 1000xg. Clear serum can be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles.

6.2 Plasma: Use EDTA, heparin, or citrate as an anticoagulant for plasma collection. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. The plasma can be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles.

6.3 Cell Culture Supernatant: Remove particulates by centrifugation for 5 minutes at 500xg and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.



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## 7. Regent Preparation

**7.1 Wash Buffer (1X):** If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 30 mL of Wash Buffer Concentrate(20X) to 570 mL deionized or distilled water to prepare 1X Wash Buffer.

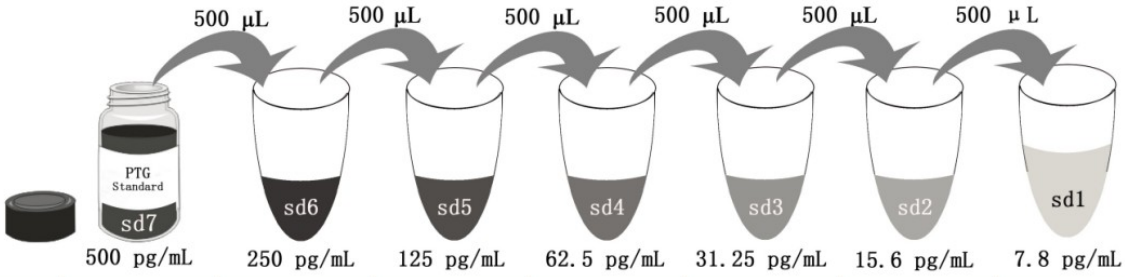
**7.2 Antibody Cocktail (1X):** Dilute 100X capture antibody and 100X HRP-conjugated detection antibody using Detection Diluent prior to assay. Suggested 1:100 dilution: 50 µL 100X capture antibody + 50 µL 100X Detection Antibody, HRP-conjugated + 4,900 µL Detection Diluent. Mix gently but thoroughly.

**7.3 Sample Dilution:** Different samples should be diluted with corresponding Sample Diluent, samples may require further dilution if the readout values are higher than the highest standard OD reading. Variations in sample collection, processing and storage may affect the results of the measurement.

Recommended Dilution for different sample types: 1:2 is recommended for rat serum and plasma. 1:2 or 1:4 is recommended for cell culture supernatant.

**7.4 Standard Serial Dilution:**

Add 2 mL Sample Diluent PT 3 in protein standard.




| Add # µL of Standard diluted in the previous step | — | 500 µL | 500 µL | 500 µL | 500 µL | 500 µL | 500 µL |
|---|---|---|---|---|---|---|---|
| # µL of Sample Diluent PT 3 | **2000 µL** | 500 µL | 500 µL | 500 µL | 500 µL | 500 µL | 500 µL |
| | "sd7" | "sd6" | "sd5" | "sd4" | "sd3" | "sd2" | "sd1" |



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## 8. Assay Procedure Summary

**Bring all reagents to room temperature before use (Detection antibody, HRP-conjugated can be used immediately). To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.**

8.1 Preset the layout of the microplate, including control group,standard group and sample group,take out the required number of microplate strips and return excess strips to the foil pouch containing the drying reagent pack and reseal; store at 4°C immediately. Microplate strips should be used in one week.

8.2 Add 50 µL standard or sample to appropriate wells.To avoid high background always add samples or standards to the well before the addition of the antibody cocktail.

8.3 Add 50 µL 1X Antibody Cocktail solution (refer to Reagent Preparation7.2) to each well. Seal plate with cover seal and Incubate at 37°C on a microplate thermostatic shaker set at 400 rpm for 1 hour .

8.4 Wash

1) Gently remove the cover seal. Discard the liquid from wells by aspirating or decanting. Remove any residual solution by tapping the plate a few times on fresh paper towels.

2) Wash 4 times with 1X Wash Buffer, using at least 350-400 µL per well. Following the last wash, firmly tap plates on fresh towels 10 times to remove residual Wash Buffer. Avoid getting any towel fibers in the wells or wells drying out completely.

8.5 Add 100 µL TMB substrate solution to each well, protected from light. Incubate at 37°C on a microplate thermostatic shaker set at 400 rpm for 15 to 20 minutes. (Substrate Solution should remain colorless until added to the plate.)

8.6 Add 100 µL Stop Solution to each well in the same order as addition of the TMB substrate . NB: Avoid skin and eye contact with the Stop solution.

8.7 Read results immediately on a microplate reader at a wavelength of 450 nm. If possible, perform a double wavelength readout (450 nm and 630 nm).

8.8 Data analysis: Calculate the average of the duplicate readings (OD value) for each standard and sample, and subtract the average of the zero standard absorbance. Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis, four-parameter logistic curve- fit (4-PL) analysis is recommend. If the samples have been diluted, the fitting result must be multiplied by the dilution factor used.

## Procedure summary



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Add 50 µL standard or sample to appropriate wells

↓

Add 50 µL antibody cocktail solution (1x) to each well

↓

Incubate at 37 °C on a microplate thermostatic shaker set at 400 rpm for 1 hour

↓

Aspirate and wash each well four times with 400µL 1 x Wash Buffer

↓

Add 100 µL TMB substrate solution to each well

↓

Incubate at 37 °C on a microplate thermostatic shaker set at 400 rpm for 15minutes

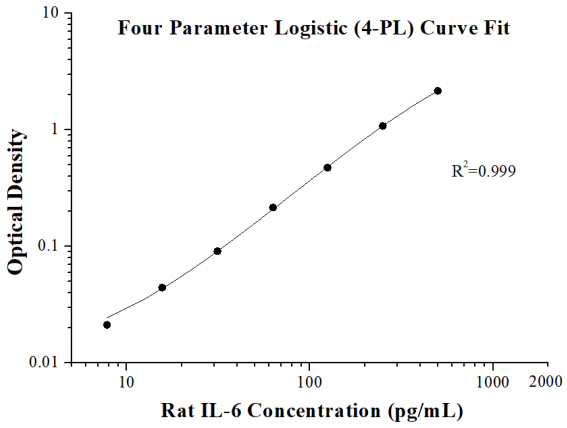
↓

Add 100 µL Stop Solution to each well and Read OD immediately

## 9. Validation Data

### 9.1 Standard curve

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.




| (pg/mL) | O.D | Average | Corrected |
|---|---|---|---|
| 0 | 0.0021<br>0.0025 | 0.0023 | - |
| 7.8 | 0.0232<br>0.0237 | 0.02345 | 0.0212 |
| 15.6 | 0.0463<br>0.0469 | 0.0466 | 0.0443 |
| 31.25 | 0.0929<br>0.094 | 0.09345 | 0.0912 |
| 62.5 | 0.2145<br>0.2218 | 0.21815 | 0.2159 |
| 125 | 0.4762<br>0.4783 | 0.4773 | 0.47495 |
| 250 | 1.0956<br>1.0726 | 1.0841 | 1.0818 |
| 500 | 2.1838<br>2.1457 | 2.16475 | 2.16245 |



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## 9.2 Precision

**Intra-assay Precision** (Precision within an assay) Three samples of known concentration were tested 8 times on one plate to assess intra-assay precision.

**Inter-assay Precision** (Precision between assays) Three samples of known concentration were tested in 16 separate assays to assess inter-assay precision.

| Intra-assay Precision | | | | |
|---|---|---|---|---|
| Sample | n | Mean (pg/mL) | SD | CV% |
| 1 | 8 | 2,099.6 | 29.9 | 1.4 |
| 2 | 8 | 519.9 | 11.6 | 2.2 |
| 3 | 8 | 271.9 | 5.6 | 2.1 |

| Inter-assay Precision | | | | |
|---|---|---|---|---|
| Sample | n | Mean (pg/mL) | SD | CV% |
| 1 | 16 | 2,091.1 | 32.9 | 1.6 |
| 2 | 16 | 521.2 | 10.2 | 1.9 |
| 3 | 16 | 273.1 | 7.7 | 2.8 |

## 9.3 Recovery

The recovery of rat IL-6 spiked to three different levels throughout the range of the assay in various matrices was evaluated.

| Sample Type | | Average% of Expected | Range (%) |
|---|---|---|---|
| Rat serum | 1:2 | 83 | 73-101 |
| | 1:4 | 108 | 102-119 |
| Cell culture supernatant | 1:2 | 90 | 85-96 |
| | 1:4 | 92 | 89-97 |

## 9.4 Sample values

**Rat serum** - Eight samples were evaluated for detectable levels of rat IL-6 in this assay. Seven measured less than the lowest rat IL-6 standard, 62.5 pg/mL. One sample read 805.4 pg/mL.

**Cell culture supernatant** - rat splenocytes (1 x $10^6$ cells/mL) were cultured for 18 hours in RPMI supplemented with 5% fetal bovine serum and stimulated with 1 µg/mL LPS. An aliquot of the cell culture supernate was removed and assayed for levels of rat IL-6.

| Condition | 18 hours (pg/mL) |
|---|---|
| Unstimulated | 15.5 |
| Stimulated | 2,868.5 |

## 9.5 Sensitivity

The minimum detectable dose of rat IL-6 is 11.5 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.



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## 9.6 Linearity

To assess the linearity of the assay, rat serum samples were spiked with high concentrations of rat IL-6 and diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay. Cell culture supernatant samples were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.

| | | Rat serum | Cell culture supernatant |
|---|---|---|---|
| 1:2 | Average% of Expected | 71 | 100 |
| | Range (%) | 70-72 | - |
| 1:4 | Average% of Expected | 102 | 107 |
| | Range (%) | 99-106 | 106-107 |
| 1:8 | Average% of Expected | 115 | 120 |
| | Range (%) | 109-121 | 117-123 |
| 1:16 | Average% of Expected | 123 | 126 |
| | Range (%) | 117-129 | 125-126 |

## 9.7 Specificity

This assay recognizes natural and recombinant rat IL-6.

The following factors prepared at 50 ng/mL were assayed and exhibited no cross-reactivity or interference.

Rat:

GM-CSF/CSF2

IL-1α

IL-4

IL-2

TNF-alpha

IL-1 beta

IL-4

## 10. References

1. Scheller J. et al. (1988). Behring Inst Mitt. Behring Inst Mitt. (83):40-7.

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