# ab285343 – Human IL-6 ELISA Kit

For the quantitative measurement of IL-6 in human serum, plasma, urine, cell culture samples, and other biological fluids.
For research use only - not intended for diagnostic use.

**For overview, typical data and additional information please visit:**
http://www.abcam.com/ab285343

## Storage and Stability

On receipt entire assay kit should be stored at 4°C, protected from light. Use kit within 6 months from the date of shipment.

## Materials Supplied

| Item | Quantity | Storage Condition |
|---|---|---|
| Micro ELISA Strip-Plate | 1 unit | 4°C |
| Standard (96 pg/mL) | 0.5 mL | 4°C |
| Standard Diluent | 6 mL | 4°C |
| HRP-Conjugate Reagent | 10 mL | 4°C |
| Sample diluent | 6 mL | 4°C |
| Chromogen Solution A | 6 mL | 4°C |
| Chromogen Solution B | 6 mL | 4°C |
| Stop Solution | 6 mL | 4°C |
| 20X Wash buffer | 25 mL | 4°C |
| Plate sealers | 2 units | 4°C |

## Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 nm
- 37°C incubator
- Precision pipettes with disposable tips
- Deionized or distilled water
- Clean Eppendorf tubes for preparing standards or sample dilutions
- Absorbent paper

## Reagent Preparation

- Prepare reagents within 30 minutes before the experiment.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.

Wash Buffer: Dilute the concentrated washing buffer (20X) with distilled water.

## Standard Preparation

- Ten wells are set for standards in a Micro Elisa strip plate.

1. In Well 1 and Well 2, 50 µl Standard solution and 50 µl Standard Dilution buffer are added and mixed well.
2. In Well 3 and Well 4, 50 µl solution from Well 1 and Well 2 are added respectively.
3. Then 50 µl Standard Dilution buffer are added and mixed well.
4. In Well 5 and Well 6, 50 µl solution from Well 3 and Well 4 are added respectively. Then 50 µl Standard Dilution buffer are added and mixed well.
5. In Well 7 and Well 8, 50 µl solution from Well 5 and Well 6 are added respectively. Then 50 µl Standard Dilution buffer are added and mixed well.
6. In Well 9 and Well 10, 50 µl solution from Well 7 and Well 8 are added respectively. Then 50 µl Standard Dilution buffer are added and mixed well. 50 µl solution is discarded from Well 9 and Well 10.
7. After dilution, the total volume in all the wells are 50 µl and the concentrations are 48 pg/ml, 24 pg/ml, 12 pg/ml, 6 pg/ml and 3 pg/ml, respectively.

## Sample Preparation

Sample extraction and ELISA assay should be performed as soon as possible after sample collection. If ELISA assay cannot be performed immediately, samples can be stored at -20°C. Avoid multiple freeze-thaw cycles. Samples with $NaN_3$ should be avoided for this assay.

Serum:
After collection of the whole blood, allow the blood to clot by leaving it undisturbed at room temperature. This usually takes 10- 20 minutes. Remove the clot by centrifuging at 2,000-3,000 rpm for 20 minutes. If precipitates appear during reservation, the sample should be centrifuge again.

Plasma:
Collect the whole blood into tubes with anticoagulant (EDTA or citrate). After incubated at room temperature for 10-20 minutes, tubes are centrifuged for 20 min at 2,000-3,000 rpm. Collect the supernatant carefully as plasma samples. If precipitates appear during reservation, the sample should be centrifuge again.

Urine:
Collect urine into aseptic tubes. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. If precipitates appear during reservation, the sample should be centrifuge again. The preparation procedure of cerebrospinal fluid and pleuroperitoneal fluid is the same as that of urine sample.

Cell Samples: If you want to detect the secretions of cells, collect culture supernatant into aseptic tubes. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. If you want to detect intracellular components, dilute the cells to 1X100/ml with PBS (pH 7.2-7.4). The cells were destroyed to release intracellular components by repeated freezing and thawing. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. If precipitates appear during reservation, the sample should be centrifuge again.

Tissue Samples: Tissue samples are cut, weighed, frozen in liquid nitrogen and stored at -80°C for future use. The tissue samples were homogenized after adding PBS (pH 7.4). Samples should be operated at 4°C. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. Aliquot the supernatant for ELISA assay and future use.

**Δ Note:** *End user should estimate the concentration of the target protein in the test sample first, and select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit.*

## Assay Protocol

Bring all reagents and samples to room temperature 30 minutes prior to the assay.
It is recommended that all standards and samples be run at least in duplicate.
A standard curve must be run with each assay.

1. Prepare all reagents, samples and standards.
2. In sample wells, add 40 µl Sample dilution buffer and 10 µl samples are added (dilution factor is 5). Leave a well empty as blank control. Samples should be loaded onto the bottom without touching the well wall. Mix well with gentle shaking.
3. Incubate 30 min at 37°C after sealed with Closure plate membrane.

4. Remove plate sealer, aspirate and refill with the wash solution. Discard the wash solution after resting for 30 seconds. Repeat the washing procedure for 5 times.
5. Add 50 µl HRP-Conjugate reagent to each well except the blank control well. Incubate 30 min at 37°C.
6. Washing as described in Step 4.
7. Add 50 µl Chromogen Solution A and 50 µl Chromogen Solution B to each well, mix with gently shaking and incubate at 37°C for 15 minutes in dark.
8. Add 50 µl stop solution to each well to terminate the reaction. The color in the well should change from blue to yellow.
9. Read absorbance O.D. at 450nm within 15 minutes after adding stop solution. The OD value of the blank control well is set as zero.

## Calculation:

Known concentrations of Human IL-6 Standard and its corresponding reading OD is plotted respectively. The concentration of Human IL-6 in sample is determined by plotting the sample's O.D. on the X-axis. The original concentration is calculated by multiplying the dilution factor.

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
www.abcam.com/protocols/the-complete-elisa-guide

**For technical support contact information, visit**: www.abcam.com/contactus

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