# ab270883 – Human IL-2 SimpleStep ELISA® Kit

For the quantitative measurement of IL-2 in human serum, plasmas, and cell culture supernatant.
For research use only - not intended for diagnostic use.
**For overview, typical data and additional information please visit: www.abcam.com/ab270883**

**Storage and Stability:** Store kit at 2-8°C immediately upon receipt. Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Standard Preparation and Reagent preparation sections.

## Materials Supplied

| Item | Quantity | Storage Condition |
|---|---|---|
| Human IL-2 Capture Antibody 10X | 600 µL | +4°C |
| Human IL-2 Detector Antibody 10X | 600 µL | +4°C |
| Human IL-2 Lyophilized Recombinant Protein | 2 Vials | +4°C |
| Antibody Diluent 4BI | 6 mL | +4°C |
| Sample Diluent 50BP | 20 mL | +4°C |
| Sample Diluent NS | 12 mL | +4°C |
| Wash Buffer PT 10X | 20 mL | +4°C |
| TMB Development Solution | 12 mL | +4°C |
| Stop Solution | 12 mL | +4°C |
| SimpleStep Pre-Coated 96-Well Microplate | 96 Wells | +4°C |
| Plate Seal | 1 | +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 or 600 nm.
Method for determining protein concentration (BCA assay recommended).
Deionized water.
Multi- and single-channel pipettes.
Tubes for standard dilution.
Plate shaker for all incubation steps.
Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

## Reagent Preparation

Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.
Sample Diluent 50BP may contain precipitate, this is normal. If precipitate is not dissolved by gentle mixing, the precipitate may be dissolved by gentle warming and mixing at 37°C for 10 minutes. If precipitate remains, gently spin down and avoid visible precipitates when pipetting.
Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations

**1X Wash Buffer PT:**

Prepare 1X Wash Buffer PT by diluting Wash Buffer PT 10X with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL Wash Buffer PT 10X with 45 mL deionized water. Mix thoroughly and gently.

**Antibody Cocktail:**
Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent 4BI. To make 3 mL of the Antibody Cocktail combine 300 µL 10X Capture Antibody and 300 µL 10X Detector Antibody with 2.4 mL Antibody Diluent 4BI. Mix thoroughly and gently.

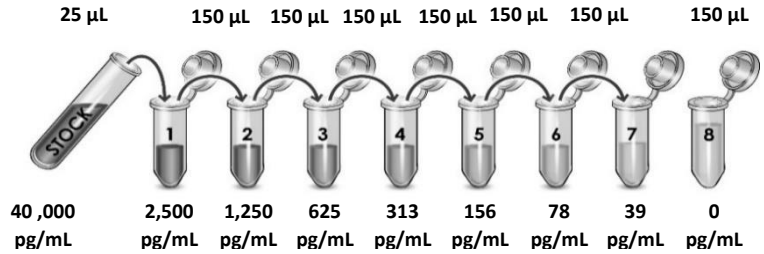
## Standard Preparation

Always prepare a fresh set of standards for every use.
Discard working standard dilutions after use as they do not store well.
The following section describes the preparation of a standard curve for duplicate measurements (recommended).

**IMPORTANT:** The sample diluent (either NS or 50BP) used to reconstitute the protein standard will depend on the samples being tested (see below). If the protein standard vial has a volume identified on the label, reconstitute the IL-2 standard by adding that volume of Diluent indicated on the label. Alternatively, if the vial has a mass identified, reconstitute the IL-2 standard by adding 500 µL Sample Diluent. Hold at room temperature for 10 minutes and mix gently. This is the 40,000 pg/mL **Stock Standard** Solution.

For **cell culture supernatant samples** reconstitute the IL-2 standard sample in **Sample Diluent NS** as above to generate the **Stock Standard** Solution.

1. Label eight tubes, Standards 1– 8.
2. Add 375 µL of Sample Diluent NS into tube number 1 and 150 µL of Sample Diluent NS into numbers 2-8.
3. Use the Stock Standard to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:

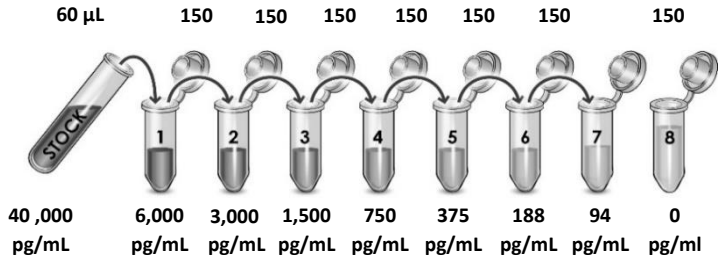



To convert sample values obtained with the kit to approximate NIBSC (86/500) units, use the following equation: NIBSC (86/500) approximate value (pg/mL) = 1.4 x SimpleStep Human IL-2 value (pg/mL).

For **serum and plasmas samples** follow these instructions reconstitute the IL-2 standard sample by adding 500 µL of **Sample Diluent 50BP** as above to generate the **Stock Standard** Solution.

1. Label eight tubes, Standards 1– 8.
2. Add 340 µL of Sample Diluent 50BP into tube number 1 and 150 µL of Sample Diluent 50BP into numbers 2-8.

3. Use the Stock Standard to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:




## Sample Preparation

| Typical Sample Dynamic Range | |
|---|---|
| **Sample Type** | **Range** |
| Serum* | ≤ 25% |
| Plasma – EDTA* | ≤ 25% |
| Plasma – Citrate* | ≤ 25% |
| Plasma – Heparin* | ≤ 25% |
| Stimulated PBMCs | 0.8 - 25% |
| RPMI 10% FBS Cell culture media* | ≤ 50% |

*Based on spiked sample

**Plasma:**
Collect plasma using citrate, EDTA or heparin. Centrifuge samples at 2,000 x g for 10 minutes. Dilute samples to at least 25% into Sample Diluent 50BP and assay. Store un-diluted plasma samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

**Serum:**
Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2,000 x g for 10 minutes and collect serum. Dilute samples to at least 25% into Sample Diluent 50BP and assay. Store un-diluted serum at -20°C or below. Avoid repeated freeze-thaw cycles.

**Cell Culture Supernatants:**
Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants and assay. Or dilute samples to at least 25% into Sample Diluent NS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

## Plate Preparation

The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.

Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.
For each assay performed, a minimum of two wells must be used as the zero control.
Differences in well absorbance or "edge effects" have not been observed with this assay.

## Assay Procedure

Equilibrate all materials and prepared reagents to room temperature prior to use.
We recommend that you assay all standards, controls and samples in duplicate

1) Prepare all reagents, working standards, and samples as directed in the previous sections.
2) Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.
3) Add 50 µL of all sample or standard to appropriate wells.
4) Add 50 µL of the Antibody Cocktail to each well.
5) Seal the plate and incubate for 60 min at room temperature on a plate shaker set to 400 rpm.
6) Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and tap gently against clean paper towels to remove excess liquid.
7) Add 100 µL of TMB Development Solution to each well and incubate for 10 minutes in the dark on a plate shaker set to 400 rpm.
   *Given variability in laboratory environmental conditions, optimal incubation time may vary between 5 and 20 minutes. Note: The addition of Stop Solution will change the color from blue to yellow and enhance the signal intensity about 3X. To avoid signal saturation, proceed to the next step before the high concentration of the standard reaches a blue color of O.D.600 equal to 1.0.*
8) Add 100 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading.
9) Alternative to 7 – 8: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Development Solution begin recording the blue color development with elapsed time in the microplate reader prepared with the following settings:

| Mode | Kinetic |
|---|---|
| Wavelength | 600 nm |
| Time | up to 20 min |
| Interval | 20 sec - 1 min |
| Shake | Shake between readings |

**Note** that an endpoint reading can also be recorded at the completion of the kinetic read by adding 100 µL Stop Solution to each well and recording the OD at 450 nm

**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