ab46027 Human IL-6 ELISA Kit

For the quantitative measurement of IL-6 in Human serum, plasma, buffered solutions and cell culture media. This product is for research use only and is not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab46027
(use abcam.cn/ab46027 for China, or abcam.co.jp/ab46027 for Japan)

Materials Supplied and Storage

Store kit at +4°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted. Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components.

Item	Quantity		Storage Condition
	1 x 96 tests	2 x 96 tests	
IL-6 Microplate (12 x 8 well strips)	96 wells	2 x 96 wells	+2-8°C
IL-6 Standard (Lyophilized)	2 vials	4 vials	+2-8°C
10X Standard Diluent Buffer	15 mL	25 mL	+2-8°C
Standard Diluent (Serum)	7 mL	2 x 7 mL	+2-8°C
Control	2 vials	4 vials	+2-8°C
Biotinylated anti-IL-6	400 µL	2 x 400 µL	+2-8°C
Biotinylated Antibody Diluent	7.5 mL	13 mL	+2-8°C
Streptavidin-HRP	2 x 5 µL	4 x 5 µL	+2-8°C
HRP Diluent	12 mL	23 mL	+2-8°C
200X Wash Buffer	10 mL	2 x 10 mL	+2-8°C
Chromogen TMB Substrate Solution	11 mL	24 mL	+2-8°C
Stop Reagent	11 mL	2 x 11 mL	+2-8°C
Adhesive Plate Seal	2 units	4 units	+2-8°C

Materials Required, Not Supplied

- Microplate reader capable of measuring absorbance at 450 nm.
- Precision pipettes to deliver 2 µL to 1 mL volumes.
- Adjustable 1-25 mL pipettes for reagent preparation.
- 100 mL and 1 liter graduated cylinders.
- Absorbent paper.
- Distilled or deionized water.
- Tubes to prepare standard or sample dilutions.
- Log-log graph paper or computer and software for ELISA data analysis.

1. Reagent Preparation

Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. Prepare only as much reagent as is needed on the day of experiment.

1.1 1X Standard Diluent Buffer: Dilute the 10X Standard Diluent Buffer 10-fold in distilled water before use.

1.2 1X Wash Buffer: Dilute the 200X Wash Buffer Concentrate 200-fold in distilled water before use. Mix gently to avoid foaming. Prepare as needed according to the following table:

Number of well strips used	Volume of 200X Wash Buffer Concentrate (mL)	Volume of distilled water (mL)
1-6	5	995
1-12	10	1,990

1.3 1X Control Solution: Lyophilized Control vials must be reconstituted with the most appropriate diluent for your test samples:
For serum and plasma samples: Use Standard Diluent (Serum).
For cell culture supernatants: Use 1X Standard Diluent Buffer.
Control vials must be reconstituted with the volume of appropriate diluent buffer that is indicated on the vial. Reconstitution of the lyophilized material with the indicated volume will yield a solution for which the IL-6 concentration is stated on the vial. Allow the reconstituted 1X Control Solution to stand for 5 minutes with gentle swirling prior to use in the assay procedure. **Do not store the 1X Control Solution after reconstitution.**

1.4 1X Biotinylated anti-IL-6: Prepare the 1X Biotinylated anti-IL-6 immediately prior to use. According to the table below, dilute the Biotinylated anti-IL-6 with the Biotinylated Antibody Diluent based on the number of wells being used in the assay procedure:

Number of well strips used	Volume of Biotinylated anti-IL-6 (µL)	Volume of Biotinylated Antibody Diluent (µL)
2	40	1,060
3	60	1,590
4	80	2,120
6	120	3,180
12	240	6,360

1.5 1X Streptavidin-HRP Solution: Add 500 µL of HRP-Diluent to the Streptavidin-HRP vial prior to use to create a Streptavidin-HRP Concentrate. Do not keep this solution for further experiments. Subsequently, prior to use in the assay procedure, prepare the 1X Streptavidin-HRP Solution by further diluting the Streptavidin-HRP Concentrate with HRP-Diluent. Use the table below to determine the volumes of each solution required to prepare the final 1X Streptavidin-HRP Solution:

Number of well strips used	Volume of Streptavidin HRP (µL)	Volume of HRP Diluent (mL)
2	30	2
3	45	3
4	60	4
6	75	5
12	150	10

2. 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).

2.1 Standard vials must be reconstituted with the appropriate diluent for your samples.
For serum and plasma samples: Use Standard Diluent (Serum).
For cell culture supernatants: Use 1X Standard Diluent Buffer.
Prepare a 200 pg/mL **Standard #1** by reconstituting with the volume indicated on the vial using the appropriate diluent buffer from above.

2.2 Label tubes #2-6 and add 100 µL of appropriate diluent into each tube.

2.3 Prepare **Standard #2** by adding 100 µL of Standard #1 to tube #2 and mix thoroughly.

2.4 Prepare **Standard #3** by adding 100 µL of Standard #2 to tube #3 and mix thoroughly.

2.5 Using the table below as a guide, prepare further serial dilutions.

2.6 Diluent Buffer serves as the zero standard (0 pg/mL).

Standard #	Volume to dilute (µL)	Diluent (µL)	Total Volume (µL)	Starting conc. (pg/mL)	Final conc. (pg/mL)
1	-	-	-	200	200
2	100	100	200	200	100
3	100	100	200	100	50
4	100	100	200	50	25
5	100	100	200	25	12.5
6	100	100	200	12.5	6.25

3. Sample Preparation

3.1 **Preparation of Plasma Samples:** Collect plasma using citrate, EDTA or heparin. Centrifuge samples at 1,000 x g for 30 minutes. Store plasma samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

3.2 **Preparation of Serum Samples:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 1,000 x g for 10 minutes and collect serum. Store serum at -20°C or below. Avoid repeated freeze-thaw cycles.

3.3 **Preparation of Cell culture Supernatants:** Centrifuge cell culture media at 1,000 x g for 10 minutes to remove debris. Collect supernatants and assay. Store samples at -20°C or below. Avoid repeated freeze-thaw cycles.

3.4 **Storage:** Aliquots of two serum samples (spiked) were stored at -20°C, 2-8°C, room temperature (RT) and at 37°C, and the IL-6 levels determined after 24 hours. There was no significant loss of IL-6 reactivity during storage at RT, 2-8°C and 37°C.

3.5 **Thawing of samples:** Three Aliquots of 2 samples (spiked) were stored at -20°C and thawed up to 5 times, and the IL-6 levels determined. There was no significant loss of IL-6 after 5 cycles of freezing and thawing.

4. 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.

4.1 Prior to use, mix all reagents thoroughly taking care not to create any foam within the vials.

4.2 Determine the number of microplate strips required to test the desired number of samples, plus appropriate number of wells needed for controls and standards. Remove sufficient microplate strips from the pouch.

4.3 Add 100 µL of each standard (see Section 2), including blank controls to the appropriate wells.

4.4 Add 100 µL of sample and 1X Control Solution to the appropriate wells.

4.5 Add 50 µL of 1X Biotinylated anti-IL-6 to all wells (see Section 1).

4.6 Cover and incubate for 1 hour at room temperature (18-25°C).

4.7 Remove the cover and wash the plate as follows:
4.7.1 Aspirate the liquid from each well.
4.7.2 Add 300 µL of 1X Wash Buffer into each well.
4.7.3 Aspirate the liquid from each well.
4.7.4 Repeat for a total of 3 washes.

4.8 Add 100 µL of 1X Streptavidin-HRP solution into all wells, including the blank wells. Re-cover and incubate at room temperature for 30 minutes.

4.9 Wash as described in Step 4.7.

4.10 Add 100 µL of Chromogen TMB substrate solution into each well and incubate in the dark for 12-15 minutes at room temperature. Avoid direct exposure to light by wrapping the plate in aluminum foil.
Δ Note: Incubation time of the substrate solution is usually determined by the microplate reader performances: many microplate readers record absorbance only up to 2.0 O.D. The O.D. values of the plate should be monitored and the substrate reaction stopped before positive wells are no longer accurately readable (maximum ~20 minutes).

4.11 Add 100 µL of Stop Reagent into each well. Results must be taken immediately after the addition of Stop Reagent, or within one hour, if the microplate is stored at 2-8°C in the dark.

4.12 Read absorbance of each well on a spectrophotometer using 450 nm as the primary wavelength and optionally 620 nm (610 nm to 650 nm is acceptable) as the reference wavelength.

5. Calculations

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

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