Li StarFish S.r.l.
Via Cavour, 35 - 20063 Cernusco S/N (MI), Italy
Tel. +39-02-92150794 - Fax. +39-02-92157285
info@listarfish.it -www.listarfish.it

ELISA for Human IL-6

Product Code: 3460-1H-6

CONTENTS, development kit for 6 plates:

Vial 1 (yellow top)
Monoclonal antibody 13A5 (150 µl)
Concentration: 1 mg/ml

Vial 2 (blue top)
Biotinylated monoclonal antibody 39C3 (80 µl)
Concentration: 1 mg/ml

Vial 3 (white top)
Streptavidin-Horseradish Peroxidase (80 µl)

Vial 4
Recombinant human IL-6 standard (1 µg)

To ensure total recovery of stated quantity, vials have been overfilled.

STORAGE:
Shipped at ambient temperature. On arrival box 1 should be stored refrigerated at 4-8°C and box 2 should be stored frozen at -20°C.

General

Intended use: For quantitative determination of native and recombinant human IL-6 in solution, e.g. cell culture supernatant.

Serum/plasma samples: Please note that determination of analyte in human serum/plasma samples by this kit requires the use of ELISA diluent (product code: 3652-D2) for dilution of samples, standard and detection antibody. The diluent prevents false positive read-outs which may be caused by interference of heterophilic antibodies commonly found in human plasma and serum. Please contact Mabtech for further information.

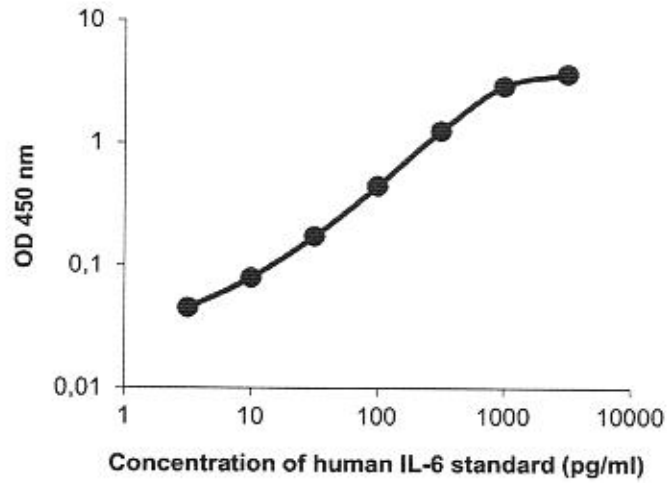
Reagents: Antibodies are supplied in sterile-filtered (0.2 µm) PBS with sodium azide (0.02%). Streptavidin-HRP is supplied in PBS with 1% BSA and 0.15% Kathon CG.

Standard range: 10-1000 pg/ml

Intra-assay variation: < 4%

Standard calibration: 1 ng of supplied standard equals 125 U of 89/548 NIBSC*-standard according to repeated calibrations. Calibration is batch-specific.

*National Institute of Biological Standards and Control, UK.



Guidelines for Human IL-6 ELISA

Day 1 1. Coat a high protein binding ELISA plate with mAb 13A5, diluted to 0.5 µg/ml in PBS, pH 7.4, by adding 100 µl/well. Incubate overnight at 4-8°C.

Day 2 2. Wash twice with PBS (200 µl/well).

3. Block plate by adding 200 µl/well of PBS with 0.05% Tween 20 containing 0.1% BSA (incubation buffer). Incubate for 1 hour at room temperature.

4. Wash five times with PBS containing 0.05% Tween.

5. Prepare IL-6 standard by reconstituting contents of vial 4 in 1 ml PBS to a concentration of 1 µg/ml. Leave at room temperature for 5 minutes and then vortex the tube. The stock solution should be stored in aliquots at -20°C for future use. We recommend the aliquots not be refrozen after initial use. For the test, prepare dilutions of the stock using the standard range as a guideline.

6. Add 100 µl/well of samples or standards diluted in incubation buffer or ELISA diluent for serum/plasma samples and incubate for 2 hours at room temperature.

7. Wash as in step 4.

8. Add 100 µl/well of mAb 39C3-biotin at 1 µg/ml in incubation buffer or ELISA diluent for serum/plasma samples. Incubate for 1 hour at room temperature.

9. Wash as in step 4.

10. Add 100 µl/well of Streptavidin-HRP diluted 1:1000 in incubation buffer. Incubate for 1 hour at room temperature. **Please note that sodium azide used in buffers will inhibit HRP activity.**

11. Wash as in step 4.

12. Add 100 µl/well of appropriate substrate solution.

13. Measure the optical density in an ELISA reader after suitable developing time. If required stop the reaction first.