

Human Interleukin-6 ELISA

Cat.n° 31670069U1 Development set

This ImmunoTools ELISA for human Interleukin-6 contains appropriate reagents sufficient for processing of 1 microplate (1 x 96 wells; 100 µl/well)

Our recombinant human IL-6 standard was calibrated against the WHO 1st International Standard (IL-6; NIBSC code 89/548) from National Institute for Biological Standards and Control, Potter Bar, Hertfordshire, UK

Specificity:

No cross reaction was detected with mouse IL-6, human Oncostatin M, human CNTF, human IL-11

Optimized standard curve range: 8 – 500 pg/ml

Content:

1x vial lyophilized anti-human IL-6 Capture-Antibody (red cap)
1x vial lyophilized anti-human IL-6 Detector-Antibody (yellow cap)
1x vial lyophilized recombinant human IL-6 standard (**50 ng rh IL-6**) (white cap)
Spin down all vials before use

Additional reagents required:

96well-Microplates
Wash-Buffer (e.g. PBS + 0.05% Tween20)
Coating-Buffer (e.g. PBS)
Blocking-Buffer (e.g. PBS + 3% BSA)
Reagent-Diluent (e.g. PBS + 3% BSA + 0.05% Tween20)
Stop-solution (e.g. 2 M H₂SO₄)
TMB-Solution
HRP-Streptavidin

Note:

All steps of incubation except HRP-Streptavidin and TMB substrate can be carried out over-night at 2 – 8°C.

Do not use solutions containing sodium azide, nor add sodium azide to the supplied reagents. Sodium azide inactivates the peroxidase.

Storage:

Protect from light!

Store at 2-8°C or longterm storage at -20°C.

Reconstituted reagents should be stored at -20°C. Please prevent repeated freeze-thaw cycles. The performance of the reagents is guaranteed until the expiration date shown on the label.

For research only. Not for use in diagnostic or therapeutic procedures.

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Human IL-6-ELISA-procedure:**Coating**

Reconstitute the lyophilized capture-antibody in 100 µl pure PBS.
 Dilute capture-antibody 1:100 in coating-buffer (100 µl capture-antibody in 10 ml PBS).
 Subsequently transfer 100 µl of this working-solution to each well and incubate 1 h at room temperature or overnight at 4°C.



Remove capture-antibody

**Blocking**

Transfer 300 µl blocking-buffer to each well and incubate 1 h at room temperature



Remove Blocking-buffer

**Addition of standard & sample**

Dilute standard & samples in reagent-diluent and transfer 100 µl of each mixture in the respective wells in duplicates.

Standard: Make serial dilutions in reagent-diluent and begin with a high standard and end with blanks.
 The standard vial of this set contains **50 ng lyophilized rh IL-6**. Reconstitute this in exactly 1 ml reagent-diluent (stock solution = 50 ng/ml) and choose a sufficient high standard working solution for your assay (e.g. prepare a 1:100 dilution for a standard curve beginning with 500 pg/ml)

Incubate at room temperature for 1 h.



Wash 5x with washing-buffer

**Addition of biotinylated detector-antibody**

Reconstitute the lyophilized detector-antibody in 100 µl blocking-buffer.
 Dilute detector-antibody 1:100 in reagent-diluent (100 µl detector-antibody in 10 ml reagent-diluent).
 Subsequently transfer 100 µl of this working-solution to each well and incubate 1 h at room temperature



Wash 5x with washing-buffer

**Addition of HRP-Streptavidin**

Dilute HRP-Streptavidin in reagent-diluent as recommended by the manufacturer.
 Subsequently transfer 100 µl of this working-solution to each well and incubate 30 minutes at room temperature



Wash 5x with washing-buffer

**Addition of TMB substrate**

Warm the solution to room temperature before use.

Add 100 µl of the TMB to each well and incubate at room temperature for approximately 5-20 minutes*
 When the enzymatic colour reaction is sufficiently proceeded stop the reaction by adding of 50 µl stop solution



Read the microplate at **450 nm**

(if wavelength correction is available, set to 540 nm, 570 nm or 630 nm as reference)

*The speed of enzymatic colour development is influenced by many factors including temperature, quality of the used HRP-conjugate and amount of bound HRP-conjugate. Therefore the incubation time is variable und specific for each test system. The development of the colour reaction has to be controlled and should be stopped at an appropriate time point.

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