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Human IL-17A ELISA development kit

Product Code: 3520-1H-6

CONTENTS, development kit for 6 plates:

Vial 1 (yellow top)
Monoclonal antibody MT44.6 (300 µl)
Concentration: 0.5 mg/ml

Vial 2 (red top)
Biotinylated monoclonal antibody MT504 (80 µl)
Concentration: 0.5 mg/ml

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

Vial 4
Recombinant human IL-17A 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-17A in solution, e.g. cell culture supernatant.

Serum/plasma samples: Please note that cytokine determinations in serum/plasma require 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 found in plasma and serum. The ELISA diluent has been validated using serum/plasma from normal healthy human blood donors. Please note that heterophilic antibody interference in samples from human subjects with various diseases or other conditions has not been assessed. Please contact Mabtech for further information.

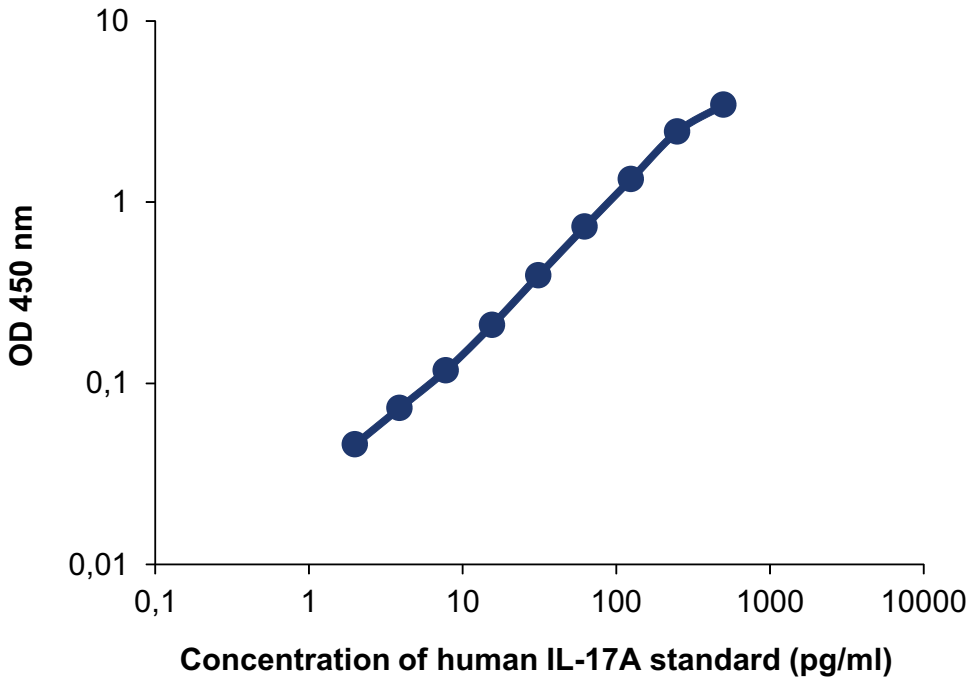
Reagents: Antibodies are supplied in sterile-filtered (0.2 µm) PBS with sodium azide (0.02%). Streptavidin-HRP is supplied in PBS with 1% BSA and 0.002% Kathon CG.

Standard range: 4-400 pg/ml

Intra-assay variation: < 5%

Standard calibration: 1 ng of supplied standard equals 12 U of 01/420 NIBSC*-standard according to repeated calibrations. Calibration is batch-specific.

*National Institute of Biological Standards and Control, UK.



Guidelines for Human IL-17A ELISA

Day 1 1. Coat a high protein binding ELISA plate with mAb MT44.6, diluted to 2 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 hIL-17A standard by reconstituting contents of vial 4 in 1 ml PBS with 1% BSA to a concentration of 1 µg/ml. Leave at room temperature for 15 minutes and then vortex the tube. The stock solution should be used immediately or 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 MT504-biotin at 0.5 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.