Human IL-12/IL-23 p40 ELISA-SET

Ref.: hp40-EIA-5

MabTag's ELISA for human Interleukin-12/23 subunit p40 (IL-12/23p40) contains appropriate reagents sufficient for processing of 5 microplates (5 x 96 wells; 100 µl/well). For research only. Not for use in diagnostic or therapeutic procedures.

Typical standard curve range: 16 – 1000 pg/ml

Detection limit: 4.9 pg/ml

Samples: Culture supernatants, serum, plasma and other body fluids.

For serum and plasma a dilution of ≥ 1:10 is recommended.

Specificity

This ELISA-Assay for quantification of TOTAL IL-12/23p40 detects all existing forms of p40 protein and cannot discriminate between these forms:
p40 as subunit of biologically active IL-12 heterodimer (p40+p35, called IL-12p70)
p40 as subunit of biologically active IL-23 heterodimer (p40+p19)
p40 monomer
p40 homodimer

Note that this assay is unable to discriminate between the active IL-12, IL-23, monomeric p40 and dimeric p40.

Content	Working dilution	Storage
1 x vial lyophilized anti-hIL-12p40 Capture-Antibody (red cap)	1:100	-20°C
1 x vial lyophilized anti-hIL-12p40 Detector-Antibody (yellow cap)	1:100	-20°C
1 x vial 50 ng lyophilized r hIL-12p40 Standard (white cap)	customer specific	-20°C
1 x vial 50 µl Poly-HRP-Streptavidin (blue or green cap)	1:1000	-20°C

Additional material required: General ELISA Reagent Pack (GenEIA-Pack-5/20) or
96well-Microplate
Coating-Buffer (e.g. PBS)
Blocking-Buffer / Reagent-Diluent (e.g. PBS + 2% BSA + 0.05% Tween20)
Wash-Buffer (e.g. PBS + 0.05% Tween20)
TMB-Substrate
Stop-solution (e.g. 2 M H_2SO_4)

MabTag GmbH, Managing director: Dr. Michael Küpper & Dr. Kai Bratke; Phone: +49-(0)162-6721349, E-mail: info@MabTag.com
Local court Oldenburg, HRB 200495, Landessparkasse zu Oldenburg, Bank Account No. 90152695, Bank Code/ BLZ 28050100;
IBAN: DE34280501000090152695; BIC/SWIFT: BRLADE21LZO; Tax-Nr.: 64/200/37879; VAT-ID-Nr.:DE248325477

!Spin down all vials before use!

Step	Incubation	Procedure
Coating Capture-antibody	≥ OVERNIGHT at room temperature	Reconstitute the lyophilized capture-antibody in 500 µl COATING-BUFFER. Dilute capture-antibody 1:100 in COATING-BUFFER (100 µl capture-antibody in 10 ml COATING-BUFFER). Subsequently transfer 100 µl of this working-solution to each well and incubate.
Remove capture-antibody completely by inverting the microplate and blotting it ***vigorously*** against clean paper towels.		
Blocking	≥ 1 Hour at room temperature	Add 300 µl BLOCKING-BUFFER to each well and incubate.
Remove BLOCKING-BUFFER completely by inverting the microplate and blotting it ***vigorously*** against clean paper towels.		
Standard & Sample	≥ 2 Hours at room temperature	Dilute standard & samples in REAGENT-DILUENT and transfer 100 µl 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 standard**. 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).
Wash 5x ***vigorously*** with WASHING-BUFFER and remove resting buffer completely by inverting the microplate and blotting it ***vigorously*** against clean paper towels.		
Detection-antibody	≥ 2 Hours at room temperature	Reconstitute the lyophilized detection-antibody in 500 µl REAGENT-DILUENT. Dilute detection-antibody 1:100 in REAGENT-DILUENT (100 µl detection-antibody in 10 ml REAGENT-DILUENT). Subsequently transfer 100 µl of this working-solution to each well and incubate.
Wash 5x ***vigorously*** with WASHING-BUFFER and remove resting buffer completely by inverting the microplate and blotting it ***vigorously*** against clean paper towels.		
Poly-HRP-Streptavidin	20-30 Min at room temperature	Dilute Poly-HRP-Streptavidin 1:1000 in REAGENT-DILUENT (10 µl in 10 ml REAGENT-DILUENT). Subsequently transfer 100 µl of this working-solution to each well and incubate.
Wash 5x ***vigorously*** with WASHING-BUFFER and remove resting buffer completely by inverting the microplate and blotting it ***vigorously*** against clean paper towels.		
Substrate solution	Up to 60 Min* at room temperature **in the dark**	Optionally warm the solution to room temperature before use. Add 100 µl of the SUBSTRATE-SOLUTION to each well and incubate. Control the development of the colour reaction continuously and stop at an appropriate time point.
Stop solution	-	When the enzymatic colour reaction is sufficiently proceeded stop the reaction by adding of 50 µl stop solution. Read the microplate at the substrate-depending wavelength. (e.g. **450** nm for TMB substrate) (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 customer-specific factors. Therefore the incubation time is variable und specific for each test system.

Note:

All incubation steps except Poly-HRP-Streptavidin and TMB substrate could be optionally carried out over-night. Do not use sodium azide-containing solutions, nor add sodium azide to the supplied reagents. Sodium azide inactivates the peroxidase.

Storage:

Specific storage conditions in the table above.

Reconstituted reagents should be stored at -20°C. Please prevent repeated freeze- thaw cycles. Stable for up to 6 months after opening when stored at -20° C. The performance of the unopened reagents is guaranteed until one year after point of delivery.

Precautions for use:

!The stop solution is an acid solution. TMB-Solution A contain H_2O_2 and tetramethylbenzidine (TMB). All Buffers and liquid antibody solutions contain 0.045% (v/v) Proclin®950 as preservative. All these compounds are harmful and cause respiratory, skin and eye irritation. Do not swallow any components of the set/kit (R22). Wear face, eye and hand clothing protection when using this material (S36). Keep out of reach of children (S2). Keep away from food, drink and animal feeding stuff (S13). !These reagents are offered for research purposes only! For *in vitro* use only. MabTag will not be held responsible for patent infringement or other violations that may occur with the use of our products.

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MabTag GmbH, Managing director: Dr. Michael Küpper & Dr. Kai Bratke; Phone: +49-(0)162-6721349, E-mail: info@MabTag.com
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