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Monkey IL-23 ELISA Antibody Pair

Catalog No: CKM110 **Size: 10 plates**

CONTENTS OF KIT:

Coating antibody (2 vials)

Product: Monoclonal antibody to monkey interleukin 23p19 (Binds the p19 subunit)
Isotype: Mouse IgG1
Production: *In vitro* using serum free medium
Purification: Ion exchange chromatography
Buffer: Prior to lyophilization: 0.25 ml PBS, 125 mM trehalose
Application: Inject 0.25 ml distilled water into the vial and dilute 100 times in PBS. The content of one vial is sufficient for five 96-well ELISA plates (480 determinations; 50 µl/well).

Detection antibody (2 vials)

Product: Biotinylated monoclonal antibody to human interleukin 23p19) (Binds the p19 subunit)
Isotype: Mouse IgG1
Production: *In vitro* using serum free medium
Purification: Protein A affinity chromatography
Labeling: With Biotin-7-NHS (N-hydroxysuccinimide)
Buffer: Prior to lyophilization: 0.5 ml PBS, 1% BSA, 125 mM trehalose
Application: Inject 0.5 ml distilled water into the vial and dilute 100 times in PBS, 0.5% BSA, 0.05% Tween-20. The content of one vial is sufficient for five 96-well ELISA plates (480 determinations; 100 µl/well).

Standard (5 vials)

Product: Recombinant interleukin 23 (IL-23p19/p40)
Application: Cytokine standard in an ELISA system
Reconstitution: Dissolve the contents of one vial by injection of 0.5 ml distilled water into the vial. Use immediately.

Conjugate (2 vials)

Product: SPP conjugate (Streptavidin-HRP)
Application: Inject 0.5 ml distilled water into the vial and dilute 100 times in PBS, 0.5% BSA, 0.05% Tween-20. The content of one vial is sufficient for five 96-well ELISA plates (480 determinations; 100 µl/well). The product should be used in conjunction with TMB substrate.

General

Sensitivity: 8 pg/ml
Specificity: Validated for detecting native rhesus macaque and cynomolgus monkey IL-23 in supernatant.
Sterility: Membrane filtered (0.2 µm)
Stability: Lyophilized SPP conjugate is stable for at least one year at -20°C, the other lyophilized products are stable for more than one year at 4°C. After reconstitution, the antibodies are stable for one year at 4°C (if kept sterile) and SPP for minimal one year at -20°C. The reconstituted standard preparation should be used immediately.

NOT FOR HUMAN USE. FOR RESEARCH ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

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Introduction

The usefulness of sandwich ELISAs (enzyme-linked immunosorbent assays) in cytokine biology is evident from the many reports published on this subject. The assay requires two antibodies (either mono- or polyclonal antibodies) that bind with high affinity to different sites on the cytokine molecule. One of the antibodies is immobilized to the wells of a 96-well microtiter plate. This so-called capture or coating antibody functions to selectively immobilize the cytokine from crude protein preparations. The second antibody (detection antibody) is labeled with biotin and binds to a different site on the cytokine molecule. Biotin allows the antibody to interact with streptavidin molecules. By using HRP (horseradish peroxidase)-labeled streptavidin, the cytokine can now quantitatively be determined by enzymatic conversion of a HRP-specific substrate to a colored product. Since many biotin molecules can be coupled to the detector antibody, several HRP molecules are bound. The enzyme activity is subsequently further increased by using complexes (polymers) of HRP-streptavidin. This results in an extreme sensitive assay with a detection limit within the low picogram range (~5 pg/ml).

Contents

- 2 vials with anti-cytokine coating antibody supplied in lyophilized form; each vial contains sufficient material for five 96-well ELISA plates.
- 2 vials with biotinylated anti-cytokine detection antibody supplied in lyophilized form; each vial contains sufficient material for five 96-well ELISA plates.
- 5 vials with cytokine standards supplied in lyophilized form.
- 2 vials with SPP conjugate (Streptavidin-HRP) supplied in lyophilized form; each vial contains sufficient material for five 96-well ELISA plates.

Required materials and reagents:

- PB stock: dissolve 96.0 g $Na_2HPO_4.2H_2O$ plus 17.5 g KH2PO4 in 1.0 L distilled water and adjust pH to 7.4
- Sterile distilled water
- 2 MH_2SO_4
- Bovine serum albumin (BSA; ELISA grade)
- 96-well ELISA plates.
- Adhesive cover slips
- Pipetting devices for the accurate delivery of volumes required for assay performance
- Plate washer: automated or manual (squirt bottle, manifold dispenser, etc.)
- Reading device for microtiter plates set at 370, 450 or 655 nm
- Tween-20 detergent
- Substrate (e.g. tetramethylbenzidine [TMB]).

Working solutions

- **PBS:** 10 ml PB stock solution and 8.8 g NaCl added to 1.0 L distilled water
- **PBST (Wash Buffer):** 0.5 ml **Tween-20** added to 1 L PBS
- **PBST-B: PBST** supplemented with 0.5% (w/v) **BSA**
- **Blocking buffer: PBS** supplemented with 1% (w/v) **BSA**

Coating antibody

- Reconstitute the contents of the vial in 250 µl distilled water and dilute 100-fold in PBS
- Bring 50 µl of diluted coating antibodies in the wells of an ELISA plate and fill up to 100 µl with PBS.
- Seal the plate with an adhesive cover slip and incubate overnight at 4°C.

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Blocking

• Remove coating antibody solution and wash the wells at least 6 times with PBST.
• Add 200 µl blocking buffer to each well.
• Seal the plate with an adhesive cover slip and incubate 1 h at 37°C or 2 h at room temperature.

Samples and standards

• Dilute samples and standards in PBST-B (see 'Cytokine standards').
• Remove blocking buffer by a vigorous 'shake-out' action but do not wash.
• Add 100 µl of diluted standards and samples to each well.
• Seal the plate and incubate for 2 h at 37°C.

Detection antibody

• Reconstitute the contents of the vial in 500 µl distilled water and dilute 100-fold in PBST-B.
• Remove standards and samples and wash the wells at least 6 times with PBST.
• Add 100 µl of diluted detector antibodies to each well.
• Seal the plate and incubate 1 h at 37°C or overnight at 4°C.

Enzyme conjugate

• Reconstitute the contents of the vial with SPP in 500 µl distilled water and dilute 100-fold in PBST-B.
• Remove detector antibody solution and wash the wells at least 6 times with PBST.
• Add 100 µl of diluted SPP conjugate to each well.
• Seal the plate and incubate 1 h at 37°C or overnight at 4°C.

Substrate

• The substrate for SPP should be prepared as follows: dissolve one TMB tablet in 1.0 ml dimethylsulfoxide (DMSO) and add this to 10 ml substrate buffer (Phosphate-Citrate buffer containing sodium perborate.
• Remove conjugate solution and wash the wells at least 6 times with PBST.
• Immediately dispense 100 µl substrate solution into each well and incubate at room temperature (15-30 minutes). In case of a positive reaction, the colorless solution will become blue that can be read at 370 nm or 655 nm. The reaction can be stopped by adding 50 µl 2M H_2SO_4 (resulting in a yellow color that can be read at 450 nm).

Cytokine standards

For maximum recovery, the vial with lyophilized cytokine standards should be reconstituted in distilled water (volume indicated on the vial) and allowed to stand for 1 minute at room temperature. Thereafter, the reconstituted cytokine standard should be used immediately (preferentially within one hour). For measuring cytokines in cell culture supernatant, both test samples and standards should be diluted in PBST-B.

Storage antibodies and standards

The vials with lyophilized coating antibodies and biotinylated detector antibodies can be safely stored in a refrigerator for a defined length of time (expiry date indicated on the vial). After reconstitution, the antibodies remain fully active for minimal 6 months at 4°C when kept sterile. However, it is strongly recommended to divide the reconstituted antibody solutions into small aliquots for single use. These aliquots should be stored at $\leq$ -20°C. Under these conditions, the antibodies are stable for at least one year.

The vials with lyophilized cytokine standard can be safely stored in a refrigerator for one year. Use the vials with cytokine standard only once.

The vials with lyophilized SPP conjugate can be safely stored at ≤ -20°C for a defined length of time (expiry date indicated on the vial). After reconstitution, the conjugate can best be divided into small aliquots for single use. These aliquots should be stored at ≤ -20°C. Under these conditions, the conjugate is stable for at least one year.

Directions for washing

Incomplete washing will adversely affect the assay. All washing must be performed with wash buffer (PBST).

Washing can be performed manually as follows: completely aspirate the liquid from all wells by gently lowering an aspiration tip (aspiration device) into each well. After aspiration, fill the wells with at least x300 µl wash buffer, then aspirate the liquid. After washing, the plate is inverted and tapped dry on absorbent paper.

Alternatively, the wash buffer may be put into a squirt bottle. If a squirt bottle is used, flood the plate with wash buffer, completely filling all wells. After washing, the plate is inverted and tapped dry on absorbent paper.

If using an automated washing device, the operating instructions should carefully be followed.

Trouble shooting

Poor consistency of replicates can be overcome by increasing the stringency of washes particularly after the incubation step with detector antibody.

High values of the blank control (optical density > 0.2) can be overcome by shortening the incubation time with the substrate solution or is caused by improper washing procedures.

Inconsistent replicates may be due to cross-contamination of wells by improper pipetting procedures.

If no signal is observed in the wells with the standards,

- try a new vial with cytokine standard
- check whether the substrate solution was properly prepared (pH should be between 5.0 and 5.5)
- verify whether the antibodies, SPP conjugate and standard preparations were properly diluted
- verify whether the SPP conjugate was properly stored (storage at room temperature can lead to a significant loss of SPP activity and consequently low OD readings)

Avoid sodium azide in wash buffers and diluents, as this is an inhibitor of peroxidase activity