cellsciences.com

Monkey IL-12/23 p40 ELISA Matched Antibody Set

Catalog No: CK1027 **Size:** 10-plate format

Specificity:	Detects free p40 monomers, p40 dimers, and the p40 subunits of heterodimeric IL-12 (p35/p40) and heterodimeric IL-23 (p19/p40) Validated for native and recombinant Rhesus Macaque IL-12, native African Green Monkey, Baboon, Cynomolgus Monkey, Pig-tailed Macaque IL-12
Sensitivity:	4 pg/mL

Introduction:

The usefulness of 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 and specificity to different epitopes of the analyte of interest. One of the antibodies is immobilized to the wells of a 96-well microtiter plate. This coating antibody, functions to selectively immobilize the analyte from crude protein preparations. The second antibody (detection antibody) is labeled with biotin and binds to another epitope of the analyte. Biotin allows the antibody to interact with streptavidin molecules. By using HRP (horseradish peroxidase)-labeled streptavidin, the analyte can be quantified using the enzymatic conversions of an HRP-specific substrate (i.e. TMB). The results are a sensitive assay with a detection limit within the low picogram range (≤ 5 pg/mL).

Reagents Provided:

Reagent	Quantity	Storage
CK1027-A Coating Antibody, Mouse mAb IgG1 anti-monkey IL-12/23 p40	2 vials	2-8 °C
CK1027-B Biotinylated Detection Antibody, Rabbit IgG, anti-monkey IL-12	2 vials	2-8 °C
CK1027-C Standard, Recombinant rhesus macaque IL-12 **6.1 ng/vial**	5 vials	2-8 °C
CK1027-D SPP Conjugate (Streptavidin-HRP)	2 vials	-20 °C in the dark

Reagents and Equipment Required:

- Bovine serum albumin (BSA; ELISA grade)
- Phosphate Buffered Saline (PBS), pH 7.4
- Pipetting device(s) for the accurate delivery of volume required for the assay performance
- Plate washer: automated or manual (squirt bottle, manifold dispenser, etc.)
- Reading device for microtiter plates with detection range suitable for selected substrate
- Sterile distilled water
- Stop Solution H_2SO_4 [recommend 0.18 M]
- TMB Substrate Solution

Cell Sciences
65 Parker Street
Unit 11
Newburyport, MA 01950
Toll Free: 888 769-1246
Phone: 978-572-107
Fax: 978-992-0298
E-mail: info@cellsciences.com
Website: www.cellsciences.com

- Tween-20 detergent
- 96-well ELISA plates with adhesive cover strips

Reagent Preparation:

- **PBS**: 5.4 mM Na_2HPO_4 + 1.3 mM KH_2PO_4 + 150 mM NaCl, pH7.4
- **Wash Buffer**: 500µL (0.5 mL) Tween-20 + 1 L PBS
- **Blocking Buffer**: PBS + 1% (w/v) BSA
- **Dilution Buffer**: PBS + 0.5% (w/v) BSA + 0.05% (w/v) Tween-20
- **Coating Antibody**: Add **250 µL** sterile distilled water to the vial. Mix gently for 15 seconds and allow to stand for 5 minutes at RT. Do not vortex. Dilute 100-fold in PBS = 50µl/well (5 x 96 well plates).
- **Detection Antibody**: Add **500 µL** sterile distilled water to the vial. Mix gently for 15 seconds and allow to stand for 5 minutes at RT. Do not vortex. Dilute 100-fold in PBS + 0.5% BSA + 0.05% Tween 20 = 100µl/well (5 x 96 well plates).
- **SPP Conjugate (Streptavidin-HRP):** Add **500 µL** sterile distilled water to the vial. Mix gently for 15 seconds and allow to stand for 5 minutes at RT. Do not vortex. Dilute 100-fold in PBS + 0.5% BSA + 0.05% Tween 20 = 100µl/well (5 x 96 well plates).
- **Standard**: add **500 µL** sterile distilled water to vial and mix gently for 15 seconds, then allow to stand for 5 minutes at RT. Do not vortex. Place on ice. Dilute immediately (within 1 hour) in Dilution Buffer to desired concentrations to be used in the standard curve range.
- In general, when TMB substrate is used, the linear portion of the curve falls within the range of 0.5 to 100pg/mL.

Assay Procedure:

Preparation of Samples:

- Samples should be clear, non-hemolyzed and non-lipemic. Excessive hemolysis and the presence of large clots or microbial growth in the sample may interfere with the performance of the test.
- Dilute Samples in Dilution Buffer, at least 2-fold.
- Avoid repeated freeze/thaw cycles of Samples.
- The diluent for the Standard and Blank control should preferentially be control serum or plasma originating from the same species. For measuring cytokines in cell culture supernatant, samples should be diluted in Dilution Buffer.

Washing:

- Incomplete washing of the wells will adversely affect the assay.
- When washing, fill wells with at least 250 µL of Wash Buffer.
- Soak for 10-20 seconds then completely empty the wells.
- Repeat these steps at least 6 times.
- After final washing, invert the plate and tap dry on absorbent paper.

Cell Sciences
65 Parker Street
Unit 11
Newburyport, MA 01950
Toll Free: 888 769-1246
Phone: 978-572-107
Fax: 978-992-0298
E-mail: info@cellsciences.com
Website: www.cellsciences.com

Method:

1. Pipette 50 µL of diluted Coating Antibody into the wells of a 96-well ELISA plate. Fill up to 100 µL with PBS.
2. Seal the plate with an adhesive cover slip and incubate overnight at 2-8° C.
3. Aspirate Coating Antibody solution and wash the wells at least 6 times with Wash Buffer.
4. Add 200 µL Blocking Buffer to each well.
5. Seal the plate with an adhesive cover slip and incubate 1 hour at 37° C.
6. Dilute Samples and Standards in Dilution Buffer.
7. Remove Blocking Buffer by a vigorous Shake-Out action but do not wash plate.
8. Add 100 µL of diluted Samples and Standards to the appropriate well(s).
9. Seal the plate and incubate for 2 hours at 37° C or overnight at 2-8° C.
10. Aspirate Samples and Standards and wash the wells at least 6 times with Wash Buffer.
11. Add 100 µL of diluted Detection Antibody to each well.
12. Seal the plate with an adhesive cover slip and incubate 1 hour at 37° C.
13. Aspirate Detection Antibody and wash the wells at least 6 times with Wash Buffer.
14. Add 100 µL of diluted SPP Conjugate to each well.
15. Seal the plate with an adhesive cover slip and incubate 1 hour at 37° C.
16. Aspirate the Conjugate and wash the wells at least 6 times with Wash Buffer.
17. Add an HRP- specific substrate solution to each well. The volume and incubation conditions depend on the type and brand of substrate used. Typically with a TMB substrate solution, the plate is incubated at room temperature in the dark for about 15 to 25 minutes. The chromogenic solution produces a blue-colored product, which can be read at 370 nm or 655 nm.
18. Stop the reaction when possible. When TMB substrate solution is used, add 100 µL of 0.18 M H_2SO_4, resulting in a yellow color that can be read at 450 nm.

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

Cell Sciences
65 Parker Street
Unit 11
Newburyport, MA 01950
Toll Free: 888 769-1246
Phone: 978-572-107
Fax: 978-992-0298
E-mail: info@cellsciences.com
Website: www.cellsciences.com