Version 10-04/24

User's Manual

Mouse Interleukin 12, Il12 ELISA Kit

REF **CKERS-Il12-036M**

Σ **10 plates**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

Mouse IL-12 ELISA development kit contains the key components required for the quantitative measurement of natural and/or recombinant IL-12 in a sandwich ELISA format within the range of 32-2,000 pg/mL. Using the ELISA protocol described below, this kit provides sufficient reagents to assay IL-12 in approximately 1,000 ELISA plate wells.

General Description

Interleukin-12 is a disulfide-linked heterodimeric protein (p70), composed of two subunits, p35 and p40, which are encoded by two different genes. Accumulating data indicate that p40 secretion precedes that of IL-12 expression. In addition, to its ability to covalently bind to p35 to form IL-12, p40 can bind to p19 to form IL-23.

Storage

Store the kit at 4°C upon receipt. For more detailed information, please download the following document on our website.

Reconstitution And Storage

Capture Antibody: 100µg of antigen-affinity purified goat anti-IL-12. Centrifuge vial prior to opening. Reconstitute in 1.0 ml sterile water for a concentration of 100µg/ml. Following reconstitution the Capture antibodies may be stored at 2-8°Cfor up to 6 months. For long term storage it is recommended to aliquot into working volumes and store at -70°C in a manual defrost freezer.

Detection Antibody: 25 µg of biotinylated antigen-affinity purified goat anti-IL-12. Centrifuge vial prior to opening. Reconstitute in 0.25 ml sterile water for a concentration of 100µg/ml. Following reconstitution the Detection antibodies may be stored at 2-8°C for up to 6 months. For long term storage it is recommended to aliquot into working volumes and store at -70°C in a manual defrost freezer.

Mouse IL-12 Standard: 1 µg of recombinant IL-12. Centrifuge vial prior to opening. Reconstitute in 1.0 ml sterile water for a concentration of 1 µg/ml. The Standard may be stored at 2-8°C for one month or aliquoted and stored at -70°C for up to three months in a manual defrost freezer.

UltraAvidin-HRP Conjugate: 40µl vial. Upon receipt, UltraAvidin-HRP conjugate should be stored at 2-8°C, DO NOT FREEZE.

TMB Liquid Substrate: Aspirate and wash plate 4 times. Add 100µl of TMB HRPO Microwell Substrate Standard Kinetic One Component "Ready Use" to each well. Incubate at room temperature and monitorcolor development with an ELISA plate reader at 450 nm with wavelength correction set at 540 nm or 570 nm. The reaction may be stopped after 15–20 minutes by adding 100µl of 2 M sulfuric acid to each well.

Plate Preparation

1. Dilute to 2.0µg/ml of capture antibody and immediately add 100µl to each ELISA plate well. Seal the plate

and incubate overnight at room temperature.

2. Aspirate the wells to remove liquid and wash the plate 4 times using 300µl of wash buffer per well.
3. After the last wash invert plate to remove residual buffer and blot on paper towel.
4. Add 300µl block buffer to each well. Incubate for at least 1 hour at room temperature.
5. Aspirate and wash plate 4 times.

Assay Procedure

Standard/Sample: Dilute standard from 2, 000 pg/ml to zero in diluent. Immediately add 100µl of standard or sample to each well in duplicate and incubate at room temperature for at least 2 hours.

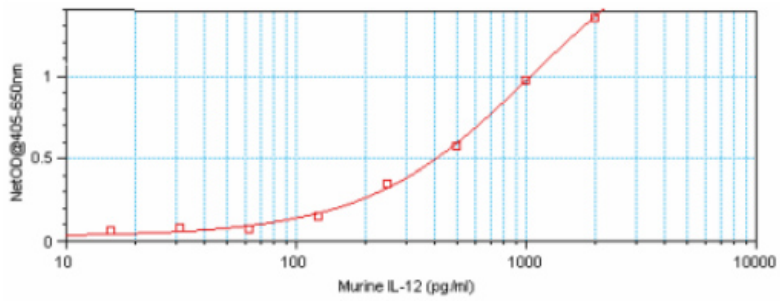
Detection: Aspirate and wash plate 4 times. Dilute a portion of detection antibody in diluent to a concentration of 50 ng/ml. Add 100µl per well and incubate at room temperature for 2 hours.

UltraAvidin-HRP Conjugate: Aspirate and wash plate 4 times. Dilute 3.0µl of avidin-HRP conjugate 1:5, 000 (this dilution factor may require some optimization) in diluent for a total volume of 15 ml. Add 100µl per well and incubate at room temperature for 30 minutes.

TMB Liquid Substrate: Add 100µl of TMB HRPO Microwell Substrate Standard Kinetic One Component "Ready Use" to each well. Incubate at room temperature for 20-30 minutes and monitor color development with an ELISA plate reader at 450nm with wavelength correction set at 540nm or 570nm. Avoid placing plates in direct light.

Stop Solution: The reaction may be stopped after 15-20 minutes by adding 100µl of 2 M sulfuric acid (Sigma Cat. # 339741) to each well. All eye, hand, face, and clothing protection should be used when working with sulfuric acid.

Typical Standard Curve



The standard curve is provided as an example only. A standard curve should be prepared with each assay.