# Mouse IL-12 (p70) ELISA Kit

**For detection and measurement of mouse interleukin 12 p70 subunit**

Catalog #02026 2 Plates

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## Product Description

The Mouse Interleukin 12 (IL-12) p70 ELISA Kit is designed for the quantitative detection and measurement of mouse IL-12 (p70) in biological fluids such as serum, plasma, and cell culture supernatants. IL-12 (p70) is a heterodimeric cytokine composed of p35 and p40 subunits; the p40 subunit is shared with IL-23. IL-12 is produced by monocytes, macrophages, dendritic cells, neutrophils, and B cells. IL-12 is a regulator of cytokine synthesis, especially IFN-γ, enhances the proliferation of T and NK cells, and stimulates the differentiation of CD4+ and CD8+ T cells.

The assay is based on the sandwich ELISA method, in which samples are added to ELISA strip plates pre-coated with capture antibodies specific for the cytokine. The captured cytokine is detected by addition of a biotinylated detection antibody, followed by streptavidin-horseradish peroxidase, which binds the biotinylated antibody. Addition of the chromogenic enzyme substrate 3,3',5,5' tetramethyl-benzidine (TMB) results in a colored product with an intensity directly proportional to the concentration of cytokine in the sample. The concentration of the cytokine is determined by comparison to serial dilutions of the cytokine standard analyzed in parallel.

## Product Information

All components listed below are stable until expiry date (EXP) on label. Once components are opened, use within 1 month. Components may be shipped at room temperature (15 - 25°C) but should be stored as indicated upon receipt.

| COMPONENT NAME | COMPONENT # | SIZE | STORAGE | DESCRIPTION |
|---|---|---|---|---|
| Mouse IL-12 & IL-12/-23 Standard | 02026B | 1 vial | Store at -20°C. | Lyophilized recombinant mouse IL-12 |
| Mouse IL-12 (p70) ELISA Plate | 02026C | 2 plates | Store at 2 - 8°C. | Plate (12 strips x 8 wells) coated with anti-mouse IL-12 (p70) antibody |
| Mouse IL-12 (p70) Biotinylated Detection Antibody | 02026D | 30 µL | Store at 2 - 8°C. | Biotinylated anti-mouse IL-12 (p70) antibody (1 mg/mL) |
| Adhesive Plate Covers | 01901 | 6 covers | Store at 2 - 8°C. | For covering plates during incubation |
| Stop Solution* | 01903 | 25 mL | Store at 2 - 8°C. | 0.18 M $H_2SO_4$ |
| TMB Substrate | 01905 | 25 mL | Store at 2 - 8°C. | 3,3',5,5' tetramethylbenzidine (TMB) enzyme substrate solution containing hydrogen peroxide |
| SA-HRP Diluent | 01907 | 25 mL | Store at 2 - 8°C. | For dilution of SA-HRP |
| ELISA Diluent | 01909 | 120 mL | Store at 2 - 8°C. | Protein-containing buffer for dilution of samples, standard, and detection antibody |
| Wash Buffer (20X) | 01910 | 120 mL | Store at 2 - 8°C. | Concentrated buffer solution for washing plates between steps |
| Standard Reconstitution Buffer C | 01913 | 1 mL | Store at 2 - 8°C. | For reconstitution of lyophilized cytokine standard |
| SA-HRP | 01914 | 30 µL | Store at 2 - 8°C. | Streptavidin-horseradish peroxidase conjugate |

*Please refer to the Safety Data Sheet (SDS) for hazard information.

## Materials Required but Not Included

- Vertical laminar flow hood certified for Level II handling of biological materials
- Microplate reader set at a wavelength of 450 nm and (optional) a second correction wavelength of 650 nm
- ELISA plate washer: Automatic (adaptable for ELISA strip plates) or manual (e.g. multi-pipette or squirt bottle)
- Micropipette (e.g. Eppendorf, Gilson) with appropriate tips
- Beakers, flasks, and graduated cylinders necessary for reagent preparations
- Tubes for standard and sample dilutions
- Timer
- Absorbent paper
- Deionized water

## Preparation of Reagents and Materials

### A. Wash Buffer

NOTE: Use clean or disposable glass or plasticware for preparation and storage of wash buffer.

Dilute Wash Buffer (20X) 1 in 20 with distilled or deionized water.

*Example: For one plate, prepare 1000 mL wash buffer by adding 50 mL Wash Buffer (20X) to 950 mL distilled or deionized water.*

### B. ELISA Plates

1. Allow plates to adjust to room temperature (15 - 25°C) before opening the bags.
2. Plan the experiment to include a standard curve and a background control (7 x 2 wells), a blank (2 wells), and sample wells.
3. Assemble the required number of strips in the plate frame. Store the remaining strips in the foil bag containing the desiccant at 2 - 8°C.
4. Wash the strips with 5 x 300 µL/well of wash buffer (prepared in section A). Ensure wash buffer is thoroughly removed from the wells by firmly tapping the plate upside down on absorbent paper.

### C. Standard Stock Solution

1. Add 0.5 mL of Standard Reconstitution Buffer C to the vial of Mouse IL-12 & IL-12/-23 Standard (final concentration 0.5 µg/mL). Let sit for 5 minutes.
2. Mix thoroughly and aliquot.

NOTE: If not used immediately, store aliquots at -20°C for up to 1 month. After thawing the aliquots, do not re-freeze.

### D. Standard Curve

NOTE: The standard curve dilutions may be prepared from freshly prepared standard stock solution or from thawed aliquots. Prepare the standard curve dilutions no more than 30 minutes prior to beginning the assay. Duplicate wells for the standard curve dilutions and the background control are recommended.

Dilute the standard stock solution (prepared in section C) to create standard curve dilutions ranging from 10 - 10,000 pg/mL according to Figure 1.

For the background control (0 pg/mL) use only ELISA Diluent.

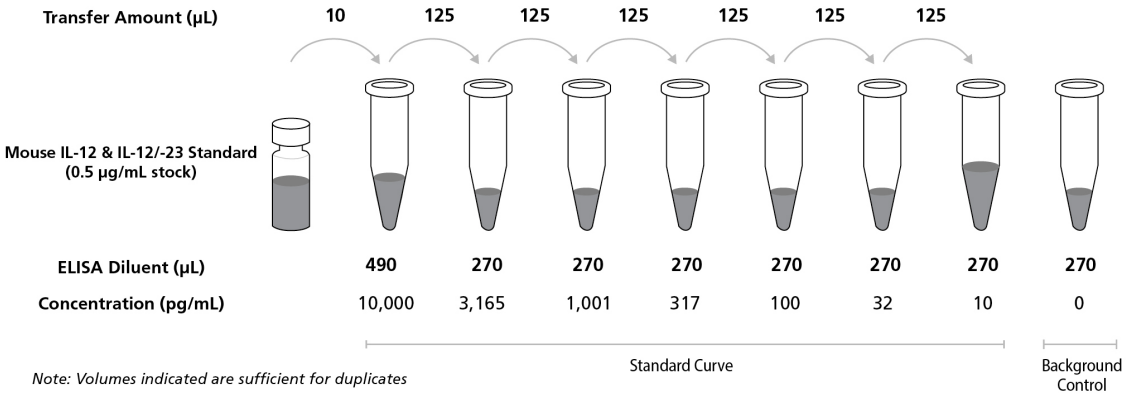


**Figure 1. Recommended Serial Dilution of Cytokine Standard**

### E. Samples

NOTE: Avoid using lipemic, hemolysed, or contaminated samples as these may yield unreliable results.

Dilute all samples at least 1:1 in ELISA Diluent in tubes, for a total minimum volume of 250 µL per dilution. Samples containing high levels of cytokine (exceeding the range of the standard curve) will require further dilution. Mix thoroughly.

NOTE: Duplicate wells for all samples are recommended. For samples with a high cytokine concentration, it is advisable to prepare several dilutions for testing. Remove any particulate matter by centrifugation or filtration prior to use.

### F. Detection Antibody

Dilute the Mouse IL-12 (p70) Biotinylated Detection Antibody 1 in 2000 in ELISA Diluent.

*Example: For one plate, add 6 µL of Mouse IL-12 (p70) Biotinylated Detection Antibody to 12 mL of ELISA Diluent (final concentration 0.5 µg/mL).*

### G. SA-HRP

Dilute SA-HRP 1 in 1000 in SA-HRP Diluent.

*Example: For one plate, add 12 µL SA-HRP to 12 mL SA-HRP Diluent.*

## Directions for Use

Please read the entire protocol before proceeding.

NOTE: Cross-contamination of reagents may invalidate assay results. Permanently labeled, dedicated, multi-channel micropipette reservoirs for reagents are recommended.

NOTE: In all washing steps, each well must be thoroughly washed and blotted dry by tapping the plate upside down on absorbent paper.

1. Bring all reagents and samples to room temperature (15 - 25°C), except the TMB Substrate, which should be kept at 2 - 8°C until use.
2. Prepare all reagents, samples, standard dilutions, and ELISA plates as described in Preparation of Reagents and Materials.
3. Add 100 µL/well of each standard curve dilution and background control (see Preparation of Reagents and Materials, section D). Leave the blank wells empty.
4. Add 100 µL/well of diluted sample (see Preparation of Reagents and Materials, section E).

   NOTE: Duplicate wells for all samples are recommended.
5. Cover the plate with an Adhesive Plate Cover and incubate at room temperature for 2 hours.
6. Wash each well with 5 x 300 µL of wash buffer (see Preparation of Reagents and Materials, section A) and blot dry.
7. Add 100 µL/well of diluted detection antibody (see Preparation of Reagents and Materials, section F). Leave blank wells empty.
8. Cover the plate with an Adhesive Plate Cover and incubate at room temperature for 1 hour.
9. Wash each well with 5 x 300 µL of wash buffer and blot dry.
10. Add 100 µL/well of diluted SA-HRP (See Preparation of Reagents and Materials, section G). Leave blank wells empty.
11. Cover the plate with an Adhesive Plate Cover and incubate at room temperature for 1 hour.
12. Wash each well with 5 x 300 µL of wash buffer and blot dry.
13. Add 100 µL/well of TMB Substrate to all wells (including blank wells).
14. Incubate at room temperature in the dark for 15 minutes.
15. Add 100 µL/well of Stop Solution to all wells (including blank wells).

    NOTE: Use care when handling Stop Solution. Please refer to the Safety Data Sheet (SDS) for hazard information.
16. Within 15 minutes of adding Stop Solution, measure the absorbance at 450 nm in a microplate reader. If possible, use a correction wavelength of 650 nm. Blank the reader using ELISA wells containing TMB Substrate and Stop Solution only.

    NOTE: Before measuring absorbance, remove any air bubbles in the wells using a small hypodermic needle or a pipette tip.
17. Calculate the average absorbance of each sample from the duplicate values. Subtract the mean absorbance value of the blank from the standard, the background control, and the sample values prior to generating the standard curve and determining the cytokine concentrations in the samples. A representative standard curve is shown in Figure 2.

    NOTE: Multiply the cytokine concentrations by the dilution factor used for each sample.

## Performance of the Assay

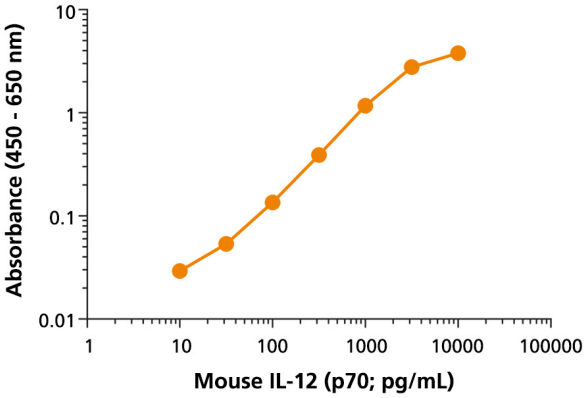


**Figure 2. Representative Standard Curve**

- Standard Range: 10 - 10,000 pg/mL.
- Sensitivity: 6 pg/mL. The lowest concentration that can be detected, but not necessarily quantified with precision and accuracy. This was determined by adding 4 standard deviations to the mean OD of background wells.
- Accuracy: No international standard exists for calibration.
- Specificity: The kit is based on a matched pair of mAbs specific for native and recombinant mouse IL-12 (p70).

**TABLE 1. Recovery**

| | SPIKE CONCENTRATION (pg/mL) | AVERAGE RECOVERY |
|---|---|---|
| Serum | 225 | 127% (range: 113 - 142) |
| | 75 | 131% (range: 120 - 139) |

NOTE: Two concentrations of standard were spiked in mouse serum. Four replicates per concentration were tested in five assays.

**TABLE 2. Intra- and Inter-Assay Precision**

| | INTRA-ASSAY | | INTER-ASSAY | |
|---|---|---|---|---|
| Sample | 1 | 2 | 1 | 2 |
| n | 8 | | 5 | |
| Mean (pg/mL) | 445.4 | 155.2 | 434.5 | 165.4 |
| Standard Deviation | 16.9 | 5.7 | 6.9 | 14.9 |
| CV% | 3.8 | 3.7 | 1.6 | 9 |

NOTE: Intra-assay and inter-assay precision were determined at two different concentrations of analyte (eight replicates per concentration in five assays).

## Troubleshooting

| PROBLEM | POSSIBLE CAUSE | RECOMMENDED ACTION |
|---|---|---|
| Absorbance values too low | Incubation time too short | Follow the directions for the multiple incubation steps outlined in Directions for Use. |
| | Undiluted Wash Buffer (20X) | Dilute Wash Buffer (20X) with distilled or deionized water as described in Preparation of Reagents and Materials, section A. |
| | Incorrect dilution of Detection Antibody and/or SA-HRP | Follow the directions for dilution of Detection Antibody and SA-HRP in Preparation of Reagents and Materials. |
| | Wash step omitted before adding SA-HRP | Follow sequence of incubation and wash steps as outlined in Directions for Use. |
| Absorbance values too high | Incubation time too long | Follow the directions for the multiple incubation steps outlined in Directions for Use. |
| | Temperature too high (> 25°C) | Perform assay at a lower temperature. If that is not possible, reduce incubation times. |

| PROBLEM | POSSIBLE CAUSE | RECOMMENDED ACTION |
|---|---|---|
| | Incorrect dilution of Detection Antibody and/or SA-HRP | Follow the directions for dilution of Detection Antibody and SA-HRP in Preparation of Reagents and Materials. |
| Low absorbance readings despite good color development in wells | Incorrect wavelength setting on the ELISA reader | Check that the measuring wavelength is set at 450 nm and that the ELISA reader has the correct filter for this wavelength. |
| High background | Insufficient washing | Ensure that each well is washed 5X with 300 µL of diluted Wash Buffer (20X). Ensure that multi-channel pipette fills and empties reproducibly. Blot wells dry before proceeding to the next step. |
| | Incorrect dilution of Detection Antibody and/or SA-HRP | Follow the directions for dilution of Detection Antibody and SA-HRP in Preparation of Reagents and Materials. |
| No signal with standard curve dilutions | Reagents not added in correct sequence | Follow sequence of incubation and wash steps as outlined in Directions for Use. |
| | Incorrect dilution of Detection Antibody and/or SA-HRP | Follow the directions for dilution of Detection Antibody and SA-HRP in Preparation of Reagents and Materials. |
| Inconsistent dose-response curve | Incubation conditions for individual wells not identical | Avoid delays during filling of assay wells; ensure that all reagents are at 15 - 25°C prior to beginning the assay. |
| Poor replicates | Insufficient washing | Ensure that each well is washed 5X with 300 µL of diluted wash buffer. Ensure that multi-channel pipette fills and empties reproducibly. Blot wells dry before proceeding to the next step. |
| | Unequal volumes in wells | Ensure that pipettes function properly. |
| | Carry-over between wells; evaporation from wells; splashing of well contents onto adhesive cover | Always use a new adhesive cover for each incubation. Ensure that each well is sealed tightly. Keep filled plates in a horizontal position and handle with caution. |
| | Samples not mixed after thawing | Vortex samples after thawing. |
| | High lipids or particulate matter in samples | Filter or centrifuge samples to pellet aggregates. Lipids may concentrate on the surface after centrifugation and may be removed. |
| | Air bubbles in well during measurement with the ELISA reader | Remove air bubbles using a small hypodermic needle or pipette tip. |
| Absorbance values of sample dilutions decrease with increasing concentration | Cytokine concentration of the sample dilutions exceeds the upper limit of the assay | Dilute the samples further and retest along with the standard curve dilutions. |

## Related Products

For a complete list of ELISA kits and related products from STEMCELL Technologies, visit our website at www.stemcell.com, or contact us at techsupport@stemcell.com.