# ab46096 – IL-2 (Interleukin-2) Mouse ELISA Kit

Instructions for Use

For the quantitative measurement of mouse IL-2 (Interleukin-2) in mouse serum, buffered solutions and cell culture supernatants.

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

Version 3b Last Updated 28 February 2024

# Table of Contents

## 1. BACKGROUND

Abcam's IL-2 (Interleukin-2) mouse *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of IL-2 in mouse serum, buffered solutions and cell culture supernatants.

A monoclonal antibody specific for IL-2 has been coated onto the wells of the microtiter strips provided. Samples, including standards of known IL-2 concentrations, control specimens or unknowns are pipetted into these wells. During the first incubation, the standards or samples and a biotinylated monoclonal antibody specific for IL-2 are simultaneously incubated. After washing, the enzyme Streptavidin-HRP, that binds the biotinylated antibody is added, incubated and washed. A TMB substrate solution is added which acts on the bound enzyme to induce a colored reaction product. The intensity of this colored product is directly proportional to the concentration of IL-2 present in the samples.

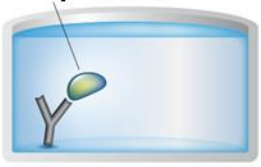
This kit will recognize both endogenous and recombinant Mouse IL-2.

## 2. ASSAY SUMMARY

Primary capture antibody

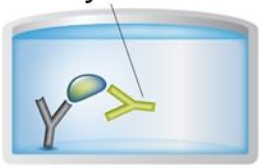
Remove appropriate number of antibody coated well strips. Equilibrate all reagents to room temperature. Prepare all the reagents, samples, and standards as instructed.

Sample



Add standard or sample to each well used.

Primary detector antibody



Add prepared Biotinylated labeled detector antibody. Incubate at room temperature

Conjugated secondary antibody

Aspirate and wash each well. Add prepared Streptavidin-HRP mix to each well. Incubate at room temperature

Substrate Colored product

Aspirate and wash each well. Add the TMB Solution to each well until color develops and then add the Stop Solution. Immediately begin recording the color development

## 3. PRECAUTIONS

**Please read these instructions carefully prior to beginning the assay.**

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

## 4. STORAGE AND STABILITY

**Store kit at +2-8ºC immediately upon receipt.**

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 9. Reagent Preparation.

## 5. MATERIALS SUPPLIED

| Item | Quantity | | Storage Condition (Before Preparation) |
|---|---|---|---|
| | 1 x 96 tests | 2 x 96 tests | |
| IL-2 Microplate (12 x 8 well strips) | 96 wells | 2 x 96 wells | +2-8ºC |
| IL-2 Standard (Lyophilized) | 2 vials | 4 vials | +2-8ºC |
| 10X Standard Diluent Buffer | 15 mL | 25 mL | +2-8ºC |
| Biotinylated anti-IL-2 | 400 µL | 2 x 400 µL | +2-8ºC |
| Biotinylated Antibody Diluent | 7.5 mL | 13 mL | +2-8ºC |
| Streptavidin-HRP | 2 x 5 µL | 4 x 5 µL | +2-8ºC |
| HRP Diluent | 12 mL | 23 mL | +2-8ºC |
| 200X Wash Buffer | 10 mL | 2 x 10 mL | +2-8ºC |
| Chromogen TMB Substrate Solution | 11 mL | 24 mL | +2-8ºC |
| Stop Reagent | 11 mL | 2 x 11 mL | +2-8ºC |

## 6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 nm.
- Precision pipettes to deliver 2 µL to 1 mL volumes.
- Adjustable 1-25 mL pipettes for reagent preparation.
- 100 mL and 1 liter graduated cylinders.
- Absorbent paper.
- Distilled or deionized water.
- Tubes to prepare standard or sample dilutions.
- Log-log graph paper or computer and software for ELISA data analysis.

## 7. LIMITATIONS

- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.
- Since exact conditions may vary from assay to assay, a standard curve must be established for every assay performed.
- Bacterial or fungal contamination of either samples or reagents or cross-contamination between reagents may cause erroneous results.
- Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.
- Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Completely empty wells before dispensing fresh 1X Wash Buffer. Do not allow wells to sit uncovered or dry for extended periods

## 8. TECHNICAL HINTS

- Kit components should be stored as indicated. All the reagents should be equilibrated to room temperature before use. Reconstituted standards should be discarded after use.
- Once the desired number of strips has been removed, immediately reseal the bag to protect the remaining strips from degradation.
- Use a clean disposable plastic pipette tip for each reagent, standard, or specimen addition in order to avoid cross-contamination; for the dispensing of the Stop Solution and substrate solution, avoid pipettes with metal parts.
- Thoroughly mix the reagents and samples before use by agitation or swirling.
- All residual washing liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells.
- The TMB solution is light sensitive. Avoid prolonged exposure to light. Also, avoid contact of the TMB solution with metal to prevent color development. Warning TMB is toxic avoid direct contact with hands. Dispose off properly.
- If a dark blue color develops within a few minutes after preparation, this indicates that the TMB solution has been contaminated and must be discarded. Read absorbances within 1 hour after completion of the assay.
- When pipetting reagents, maintain a consistent order of addition from well-to-well. This will ensure equal incubation times for all wells.
- Dispense the TMB solution within 15 minutes following the washing of the microtiter plate.

- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**

## 9. REAGENT PREPARATION

Equilibrate all reagents and samples to room temperature (18-25°C) prior to use.

### 9.1 1X Standard Diluent Buffer

Dilute the 10X Standard Diluent Buffer 10-fold in distilled water before use.

### 9.2 1X Wash Buffer

Dilute the 200X Wash Buffer Concentrate 200-fold in distilled water before use. Mix gently to avoid foaming. The 1X Wash Buffer can be prepared as needed according to the following table:

| Number of well strips used | Volume of 200X Wash Buffer Concentrate (mL) | Volume of distilled water (mL) |
|---|---|---|
| 1-6 | 5 | 995 |
| 1-12 | 10 | 1,990 |

### 9.3 1X Biotinylated anti-IL-2

Prepare the 1X Biotinylated anti-IL-2 immediately prior to use. According to the table below, dilute the Biotinylated anti-IL-2 with the Biotinylated Antibody Diluent based on the number of wells being used in the assay procedure:

| Number of well strips used | Volume of Biotinylated anti- IL-2 (µL) | Volume of Biotinylated Antibody Diluent (µL) |
|---|---|---|
| 2 | 40 | 1,060 |
| 3 | 60 | 1,590 |
| 4 | 80 | 2,120 |
| 6 | 120 | 3,180 |
| 12 | 240 | 6,360 |

### 9.4 1X Streptavidin-HRP Solution

Add 500 µL of HRP-Diluent to the Streptavidin-HRP vial prior to use to create a Streptavidin-HRP Concentrate. Do not keep this solution for further experiments.

Subsequently, prior to use in the assay procedure, prepare the 1X Streptavidin-HRP Solution by further diluting the Streptavidin-HRP Concentrate with HRP-Diluent. Use the table below to determine the volumes of each solution required to prepare the final 1X Streptavidin-HRP Solution:

| Number of well strips used | Volume of Streptavidin-HRP (µL) | Volume of HRP-Diluent (mL) |
|---|---|---|
| 2 | 30 | 2 |
| 3 | 45 | 3 |
| 4 | 60 | 4 |
| 6 | 75 | 5 |
| 12 | 150 | 10 |

## 10. STANDARD PREPARATION

Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use.

10.1 Prepare a 500 pg/mL **Standard #1** by reconstituting with the volume indicated on the vial using the 1X Standard Diluent Buffer.

10.2 Label tubes #2-6 and add 100 µL of 1X Standard Diluent Buffer.

10.3 Prepare **Standard #2** by adding 100 µL of Standard #1 to tube #2 and mix thoroughly**.**

10.4 Prepare **Standard #3** by adding 100 µL of Standard #2 to tube #3 and mix thoroughly**.**

10.5 Using the table below as a guide, prepare further serial dilutions.

10.6 1X Standard Diluent Buffer serves as the zero standard (0 pg/mL).

**Standard Dilution Preparation Table**

| Standard # | Volume to Dilute (µL) | Diluent (µL) | Total Volume (µL) | Starting Conc. (pg/mL) | Final Conc. (pg/mL) |
|---|---|---|---|---|---|
| 1 | - | - | - | 10,000 | 10,000 |
| 2 | 100 | 100 | 200 | 10,000 | 5,000 |
| 3 | 100 | 100 | 200 | 5,000 | 2,500 |
| 4 | 100 | 100 | 200 | 2,500 | 1,250 |
| 5 | 100 | 100 | 200 | 1,250 | 625 |
| 6 | 100 | 100 | 200 | 625 | 312.5 |

## 11. SAMPLE PREPARATION AND STORAGE

- **Preparation of Cell culture Supernatants**

  Centrifuge cell culture media at 1,000 x g for 10 minutes to remove debris. Collect supernatants and assay. Store samples at -20°C or below. Avoid repeated freeze-thaw cycles.

## 12. PLATE PREPARATION

- The 96 well plate strips included with this kit is supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused well strips should be returned to the plate packet and stored at 4°C.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Well effects have not been observed with this assay. Contents of each well can be recorded on the template sheet included in the Resources section.

## 13. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

13.1 Prior to use, mix all reagents thoroughly taking care not to create any foam within the vials.

13.2 Determine the number of microplate strips required to test the desired number of samples, plus appropriate number of wells needed for controls and standards. Remove sufficient microplate strips from the pouch.

13.3 Add 100 µL of each standard (see Section 10), including blank controls to the appropriate wells.

13.4 Add 50 µL of 1X Biotinylated anti-IL-2 to all wells (see Section 9).

13.5 Cover and incubate for 3 hours at room temperature (18-25°C).

13.6 Remove the cover and wash the plate as follows:

13.6.1 Aspirate the liquid from each well.

13.6.2 Add 300 µL of 1X Wash Buffer into each well

13.6.3 Aspirate the liquid from each well.

13.6.4 Repeat for a total of 3 washes.

13.7 Add 100 µL of 1X Streptavidin-HRP solution into all wells, including the blank wells. Re-cover and incubate at room temperature for 30 minutes.

13.8 Wash as described in Step 13.6.

13.9 Add 100 µL of Chromogen TMB substrate solution into each well and incubate in the dark for 10-20 minutes at room temperature. Avoid direct exposure to light by wrapping the plate in aluminum foil.
*Note:* Incubation time of the substrate solution is usually determined by the microplate reader performances: many

microplate readers record absorbance only up to 2.0 O.D. The O.D. values of the plate should be monitored and the substrate reaction stopped before positive wells are no longer accurately readable (maximum ~20 minutes).

13.10 Add 100 µL of Stop Reagent into each well. Results must be taken immediately after the addition of Stop Reagent, or within one hour, if the microplate is stored at 2-8°C in the dark.

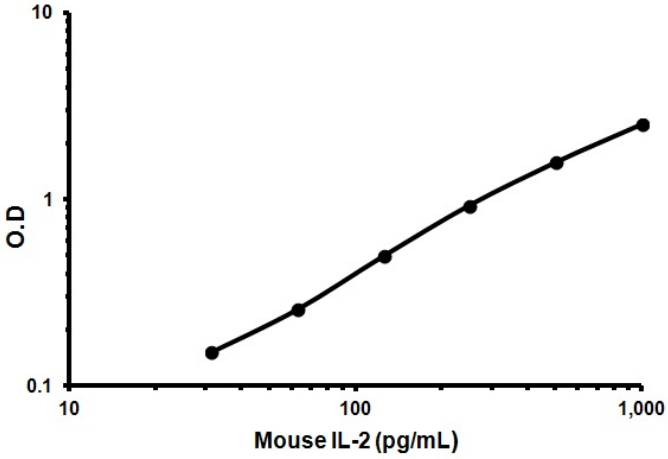
13.11 Read absorbance of each well on a spectrophotometer using 450 nm as the primary wavelength and optionally 620 nm (610 nm to 650 nm is acceptable) as the reference wavelength.

## 14. CALCULATIONS

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

## 15. TYPICAL DATA

**TYPICAL STANDARD CURVE** - Data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.




| Conc. (pg/mL) | O.D. |
|---|---|
| 1,000 | 2.51 |
| 500 | 1.572 |
| 250 | 0.926 |
| 125 | 0.495 |
| 62.5 | 0.256 |
| 31.25 | 0.151 |
| 0 | 0.025 |

## 16. TYPICAL SAMPLE VALUES

**SENSITIVITY –**

The minimum detectable dose of mouse IL-2 is <3.4 pg/mL. This has been determined by adding 3 standard deviations to the mean optical density obtained when the zero standard was assayed 32 times.

**PRECISION –Sample A**

| | Intra-Assay |
|---|---|
| n= | 10 |
| Mean (pg/mL) | 170 |
| SD | 13 |
| CV (%) | 7.8 |

**PRECISION –Sample B**

| | Inter-Assay |
|---|---|
| n= | 8 |
| Mean (pg/mL) | 137 |
| SD | 8.9 |
| CV (%) | 6.4 |

**DILUTION PARALLELISM**

A mouse serum pool containing 500 pg/mL of measured mouse IL-2 was serially diluted in mouse serum over the range of the assay. Linear regression of samples versus the expected concentration yielded a correlation coefficient of 0.99

## 17. ASSAY SPECIFICITY

This kit detects IL-2 in Mouse samples. Other species have not yet been tested with this kit

Please contact our Technical Support team for more information

## 18. TROUBLESHOOTING

| Problem | Cause | Solution |
|---|---|---|
| Poor standard curve | Inaccurate pipetting | Check pipettes |
| | Improper standards dilution | Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing |
| Low Signal | Incubation times too brief | Ensure sufficient incubation times; change to overnight standard/sample incubation |
| | Inadequate reagent volumes or improper dilution | Check pipettes and ensure correct preparation |
| Large CV | Plate is insufficiently washed | Review manual for proper wash technique. If using a plate washer, check all ports for obstructions |
| | Contaminated wash buffer | Prepare fresh wash buffer |
| Low sensitivity | Improper storage of the ELISA kit | Store the reconstituted protein at -80°C, all other assay components 4°C. Keep substrate solution protected from light. |

## 19. NOTES

## Technical Support


For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)