Mouse IL-2 ELISA Kit

Catalog Number KMC0021 (96 tests), KMC0022 (2 x 96 tests), KMC0021C (5 x 96 tests)

Pub. No. MAN0003964 **Rev.** 3.0 (31)

CAUTION! This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state, and local regulations for disposal.

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *ELISA Technical Guide* (Pub. no. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The Invitrogen™ Mouse IL-2 ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA). This assay is designed to detect and quantify the level of mouse IL-2 in mouse serum, plasma, buffered solution, or cell culture medium. The assay will recognize both natural and recombinant mouse IL-2.

Contents and storage

Upon receipt, store the kit at 2°C to 8°C.

Contents	Cat. No. KMC0021 (96 tests)
Ms IL-2 Standard; contains 0.1% sodium azide.	2 vials
Standard Diluent Buffer; contains 0.1% sodium azide	25 mL
Antibody Coated Wells, 96-well plate	1 plate
Ms IL-2 Biotin Conjugate; contains 0.1% sodium azide	6 mL
Streptavidin-HRP (100X)	0.125 mL
Streptavidin-HRP Diluent; contains 3.3 mM thymol	25 mL
Incubation Buffer; contains 0.1% sodium azide	2 × 6 mL
Wash Buffer Concentrate (25X)	100 mL
Stabilized Chromogen, Tetramethylbenzidine (TMB)	25 mL
Stop Solution	25 mL
Adhesive Plate Covers	3

Materials required but not supplied

- Distilled or deionized water
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions; beakers, flask and cylinders for preparation of reagents
- Microtiter plate reader with software capable of measurement at or near 450 nm
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)

Before you begin

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at **thermofisher.com**.
- Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.

Prepare 1X Wash Buffer

1. Dilute 16 mL of Wash Buffer Concentrate (25X) with 384 mL of deionized or distilled water. Label as 1X Wash Buffer.
2. Store the concentrate and 1X Wash Buffer in the refrigerator. Use the diluted buffer within 14 days.

Sample preparation guidelines

- Refer to the *ELISA Technical Guide* at **thermofisher.com** for detailed sample preparation procedures.
- Collect samples in pyrogen/endotoxin-free tubes.
- Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
- Avoid the use of hemolyzed or lipemic sera. If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.

Pre-dilute samples

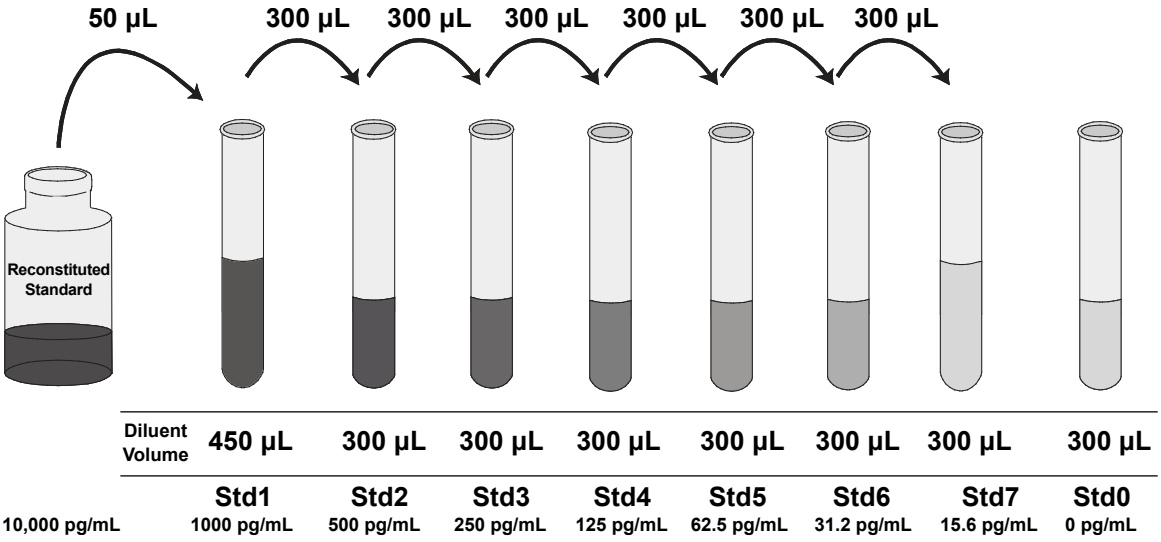
Sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application.

- Perform sample dilutions with Standard Diluent Buffer.
- Dilute samples further and reanalyze, if the sample concentrations exceed the standard curve.

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

1. Reconstitute Ms IL-2 Standard to 10,000 pg/mL with Standard Dilution Buffer. Refer to the standard vial label for instructions. Swirl or mix gently and allow the contents to sit for 10 minutes to ensure complete reconstitution. Label as 10,000 pg/mL mouse IL-2. **Use the standard within 1 hour of reconstitution.**
2. Add 50 µL Reconstituted Standard to one tube containing 450 µL Standard Diluent Buffer and mix. Label as 1,000 pg/mL mouse IL-2.
3. Add 300 µL Standard Diluent Buffer to each of 7 tubes labeled as follows: 500, 250, 125, 62.5, 31.2, 15.6 and 0 pg/mL mouse IL-2.
4. Make serial dilutions of the standard as shown in the following dilution diagram. Mix thoroughly between steps.
5. Remaining reconstituted standard should be discarded or frozen in aliquots at –80°C for further use. Standard can be frozen and thawed one time only without loss of immunoreactivity.



Prepare 1X Streptavidin-HRP solution

Note: Prepare 1X Streptavidin-HRP within 15 minutes of usage.

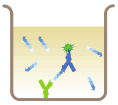
1. For each 8-well strip used in the assay, pipet 10 µL Streptavidin-HRP (100X) solution, and dispense the solution into a tube containing 1 mL of 1X Assay Buffer. Mix thoroughly.
2. Return the unused Streptavidin-HRP (100X) solution to the refrigerator.

Perform ELISA (Total assay time: 3 hours)

IMPORTANT! Perform a standard curve with each assay.

- Allow all components to reach room temperature before use. Mix all liquid reagents prior to use.
- Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store at 2°C to 8°C for future use.

1 Bind antigen



a. Add 50 µL of **Incubation Buffer** followed by 50 µL of sample (standard, serum, plasma, or tissue culture supernatant). Leave the wells for chromogen blanks empty.
b. Add 50 µL Ms IL-2 Biotin Conjugate solution into each well except the chromogen blanks.
c. Tap the side of the plate to mix. Cover the plate with a plate cover and incubate for 2 hours at 37°C.
d. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

2 Add Streptavidin-HRP

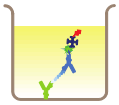
a. Add 100 µL 1X Streptavidin-HRP solution (see page 2) into each well except the chromogen blanks.
b. Cover the plate with a plate cover and incubate for 30 minutes at room temperature.
c. Thoroughly aspirate the solution from the wells and wash wells 4 times with 1X Wash Buffer.

3 Add Stabilized Chromogen

a. Add 100 µL Stabilized Chromogen to each well. The substrate solution begins to turn blue.
b. Incubate for 30 minutes at room temperature in the dark.

Note: TMB should not touch aluminum foil or other metals.

4 Add Stop Solution



Add 100 µL Stop Solution to each well. Tap the side of the plate to mix. The solution in the wells changes from blue to yellow.

Read the plate and generate the standard curve

1. Read the absorbance at 450 nm. Read the plate within 2 hours after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A 4 parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

   Note: Dilute samples producing signals greater than the upper limit of the standard curve in Standard Diluent Buffer and reanalyze. Multiply the concentration by the appropriate dilution factor.

Performance characteristics

Standard curve example

The following data were obtained for the various standards over the range of 0 to 1,000 pg/mL mouse IL-2.

Standard Mouse IL-2 (pg/mL)	Optical Density (450 nm)
1,000	3.00
500	2.20
250	1.23
125	0.64
62.5	0.35
31.2	0.20
15.6	0.11
0	0.02

Inter-assay precision

Samples were assayed 30 times in multiple assays to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	118.5	365.4	749.7
Standard Deviation	6.7	16.3	41.7
% Coefficient of Variation	5.6	4.4	5.5

Intra-assay precision

Samples of known mouse IL-2 concentration were assayed in replicates of 32 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	114.4	391.7	771.9
Standard Deviation	5.5	10.2	32.8
% Coefficient of Variation	4.8	2.6	4.3

Expected values

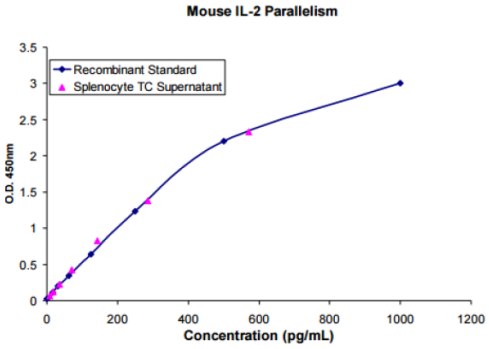
Sixteen sera, sixteen plasma (heparin) samples were evaluated in this assay. All samples measured <15.6 pg/mL (the lowest mouse IL-2 standard).

Mouse splenocytes were cultured under the following conditions, and the culture supernatants were assayed for mouse IL-2 released.

Sample	Average (pg/mL)
Con-A (5 µg/mL) 6 hr	605
PMA (50 ng/mL), Ionophore (250 ng/mL) 6 hr	555
PMA (50 ng/mL), Ionophore (250 ng/mL) 24 hr	2,724
LPS (1 µg/mL)	827

Parallelism

Natural mouse IL-2 was serially diluted in Standard Diluent Buffer. The optical density of each dilution was plotted against the standard curve. The standard accurately reflects natural mouse IL-2 content in samples.



Sensitivity

The analytical sensitivity of the assay is <4 pg/mL mouse IL-2. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 30 times, and calculating the corresponding concentration.

Specificity

Buffered solutions of a panel of substances at 10,000 pg/mL were assayed with the Mouse IL-2 ELISA Kit. The following substances were tested and found to have no cross-reactivity: **Mouse** IL-1β, IL-3, IL-4, IL-6, IL-10, IFN-γ, MCP-1, TNF-α; Rat IL-1α, IL-1β, IL-2, IL-4, IL-10, IFN-γ, TNF-α; **Human** IL-2, IL-5, IL-12, GM-CSF, RANTES.

Recovery

The recoveries of mouse IL-2 added to mouse serum, plasma, and cell culture media containing 1% fetal bovine serum, and cell culture media containing 10% fetal bovine serum were measured with the Mouse IL-2 ELISA Kit.

Sample	Average % Recovery
Serum	100
Citrate plasma	100
EDTA plasma	105
Heparin plasma	105
RPMI+1% fetal bovine serum	106
RPMI+10% fetal bovine serum	105

Linearity of dilution

Mouse serum and cell culture medium containing 1% fetal bovine serum were spiked with mouse IL-2 and serially diluted in Standard Diluent Buffer over the range of the assay. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99 in both cases.

Dilution	Serum			Cell Culture		
	Measured (pg/mL)	Expected		Measured (pg/mL)	Expected	
		(pg/mL)	%		(pg/mL)	%
Neat	839.0	839.0	—	831.8	831.8	—
1/2	481.8	419.5	114.9	345.2	415.9	83.0
1/4	246.2	209.8	117.4	178.6	208.0	85.9
1/8	112.1	104.9	106.9	107.9	104.0	103.7
1/16	50.1	52.4	95.5	44.8	52.0	86.1
1/32	24.4	26.2	93.0	23.7	26.0	91.2
1/64	13.3	13.1	101.7	12.0	13.0	92.2
1/128	5.7	6.6	86.3	6.1	6.5	94.6

Limited product warranty

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Product label explanation of symbols and warnings

REF	Catalog Number	LOT	Batch code		Temperature limitation		Use by		Manufacturer		Consult instructions for use		Caution, consult accompanying documents

Manufacturer's address: Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria

The information in this guide is subject to change without notice.