Performance characteristics, continued

Intra-assay precision

Samples of known Ms 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
SD	5.5	10.2	32.8
%CV	4.8	2.6	4.3

SD = Standard Deviation; CV = Coefficient of Variation

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
SD	6.7	16.3	41.7
%CV	5.6	4.4	5.5

SD = Standard Deviation; CV = Coefficient of Variation

Recovery

The recoveries of Ms IL-2 added to mouse serum, plasma, and tissue culture media containing 1% fetal bovine serum, and tissue culture media containing 10% fetal bovine serum were measured with the Ms 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 tissue culture medium containing 1% fetal bovine serum were spiked with Ms 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 (pg/mL)	% Expected	Measured (pg/mL)	Expected (pg/mL)	% Expected
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

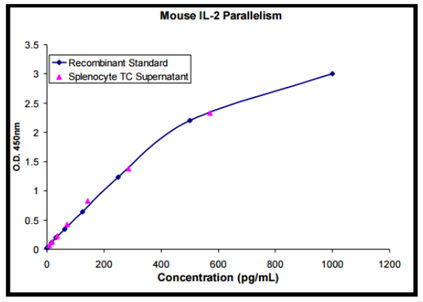
Expected values

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

Mouse splenocytes were cultured under the following conditions, and the culture supernatants were assayed for Ms 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	2724
LPS (1 µg/mL)	827

Parallelism



Natural Ms IL-2 was serially diluted in Standard Diluent Buffer. The optical density of each dilution was plotted against the standard curve. Parallelism between the natural and recombinant protein was demonstrated by the figure below and indicated that the standard accurately reflects natural Ms IL-2 content in samples.

Important licensing information

This product may be covered by one or more Limited Use Label Licenses. By use of this product, you accept the terms and conditions of all applicable Limited Use Label Licenses.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

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

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29 September 2015

invitrogen

Mouse IL-2 ELISA Kit

Catalog nos. KMC0021
KMC0022
KMC0021C

Quantity: 96 tests
192 tests
480 tests

Pub. No. MAN0003964 **Rev** 1.0

Description

The Mouse Interleukin-2 (Ms 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 Ms IL-2 in mouse serum, plasma, buffered solution, or cell culture medium. The assay will recognize both natural and recombinant Ms IL-2.

Contents and storage

The components included in the ELISA kit are listed below. Upon receipt, store the kit at 2°C to 8°C.

Components	KMC0021 96 tests	KMC0022 192 tests	KMC0021C 480 tests
Ms IL-2 Antibody Coated Wells. 96 well plate.	1 plate	2 plates	5 plates
Ms IL-2 Biotin Conjugate. Contains 0.1% sodium azide.	6 mL	12 mL	60 mL
Ms IL-2 Standard, recombinant Ms IL-2. Contains 0.1% sodium azide. Refer to vial label for quantity and reconstitution volume.	2 vials	4 vials	10 vials
Incubation Buffer. Contains 0.1% sodium azide.	2 × 6 mL	2 × 6 mL	5 × 6 mL
Wash Buffer Concentrate (25X).	100 mL	100 mL	200 mL
Standard Diluent Buffer. Contains 0.1% sodium azide.	25 mL	50 mL	125 mL
Streptavidin-HRP (100X). Contains 3.3 mM thymol.	0.125 mL	2 × 0.125 mL	5 × 0.125 mL
Streptavidin-HRP Diluent. Contains 3.3 mM thymol.	25 mL	25 mL	75 mL
Stabilized Chromogen, Tetramethylbenzidine (TMB).	25 mL	25 mL	75 mL
Stop Solution.	25 mL	25 mL	75 mL
Adhesive Plate Covers.	3	4	15

Materials required but not provided

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

General Guidelines

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at **thermofisher.com/techresources** for details prior to starting the procedure.
- Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

For Research Use Only. Not for use in diagnostic procedures.

Manufacturing Site • 7335 Executive Way • Frederick • MD 21704 • E-mail: **techsupport@lifetech.com**

Prepare 1X Wash Buffer

1. Allow the Wash Buffer Concentrate (25X) to reach room temperature and mix to redissolve any precipitated salts.
2. Dilute 16 mL of Wash Buffer Concentrate (25X) with 384 mL of deionized or distilled water. Label as 1X Wash Buffer.
3. Store the concentrate and 1X Wash Buffer in the refrigerator. Use the diluted buffer within 14 days.

Sample preparation guidelines

- 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.

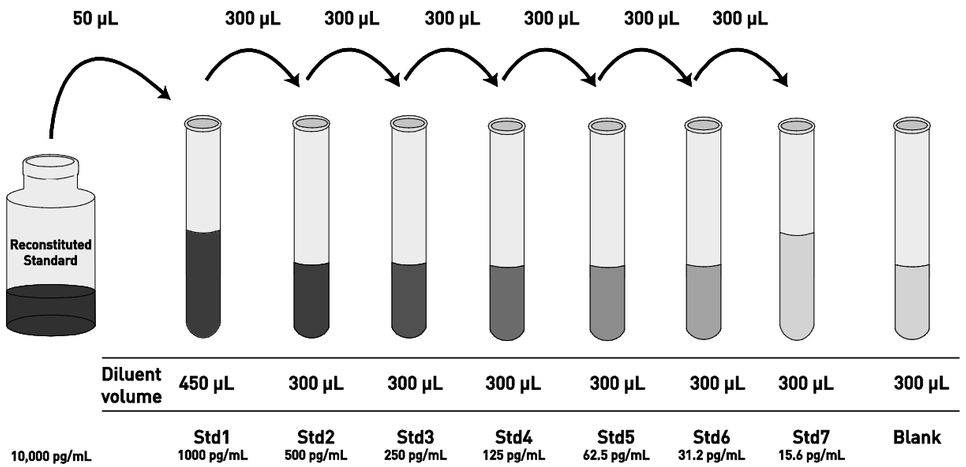
Sample dilution guidelines

- If the sample concentrations exceed the standard curve, dilute samples further and reanalyze.
- Because conditions may vary, it is recommended that each investigator determine the optimal dilution to be used for each application.

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

1. Reconstitute Ms IL-2 Standard to 10,000 pg/mL with Standard Diluent 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 Ms 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 1000 pg/mL Ms IL-2.
3. Add 300 µL Standard Diluent Buffer to each of 6 tubes labeled as follows: 500, 250, 125, 62.5, 31.2, and 15.6 pg/mL Ms IL-2.
4. Make serial dilutions of the standard as described below in the 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.

The Streptavidin-HRP (100X) is in 50% glycerol, which is viscous. To ensure accurate dilution:

1. Allow Streptavidin-HRP (100X) to reach room temperature and mix gently.
2. For each 8-well strip used in the assay, pipet 10 µL Streptavidin-HRP (100X) solution, wipe the pipette tip with a clean absorbent paper to remove any excess solution, and dispense the solution into a tube containing 1 mL of Streptavidin-HRP Diluent. Mix thoroughly.
3. Return the unused Streptavidin-HRP (100X) solution to the refrigerator.

ELISA procedure

Allow all reagents to reach room temperature before use. Mix all liquid reagents prior to use. **Total assay time is 3 hours.**

IMPORTANT! Perform a standard curve with each assay.

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 to 8°C for future use.

Bind antigen

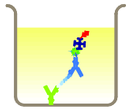
1. Add 50 µL of Incubation Buffer followed by 50 µL of sample (standard, serum, plasma, or tissue culture supernatant). Add 50 µL of Standard Diluent Buffer to the zero standard wells. Leave wells for chromogen blanks empty.
2. Add 50 µL Ms IL-2 Biotin Conjugate solution into each well except the chromogen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

Add Streptavidin-HRP

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

Add chromogen

8. Add 100 µL Stabilized Chromogen to each well. The substrate solution will begin to turn blue.
9. Incubate for 30 minutes at room temperature **in the dark**.
   Note: TMB should not touch aluminum foil or other metals.



Add stop solution

10. Add 100 µL of Stop Solution to each well. Tap side of the plate gently 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 30 minutes after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A four 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 that of the highest standard 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 0-1000 pg/mL Ms IL-2.

Standard Ms IL-2 (pg/mL)	Optical Density (450 nm)
1000	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

Specificity

Buffered solutions of a panel of substances at 10,000 pg/mL were assayed with the Ms 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.

Sensitivity

The minimum detectable dose of Ms IL-2 is <4 pg/mL. 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.