Performance characteristics, continued

Intra-assay precision

Samples of known Ms IFN-γ concentration were assayed in replicates of 16 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	145.0	37.0	10.9
SD	5.1	1.2	0.3
%CV	3.5	3.1	3.1

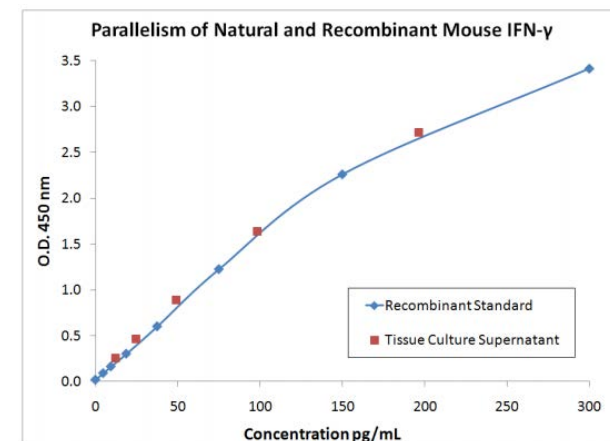
SD = Standard Deviation; CV = Coefficient of Variation

Recovery

The recovery of Ms IFN-γ added to mouse serum averaged 88.9%. The recovery of Ms IFN-γ added heparin, and citrate mouse plasma averaged 106.0% and 96.6%, respectively. The recovery of Ms IFN-γ added to tissue culture medium containing 10% fetal bovine serum averaged 100.3%.

Parallelism

Natural IFN-γ from mouse splenocytes stimulated with 10 µg/mL PHA was serially diluted in Standard Diluent Buffer. The optical density of the expected value of each dilution was plotted against the Ms IFN-γ standard curve. Parallelism was demonstrated by the figure below and indicated that the standard accurately reflects Ms IFN-γ content in samples.



Inter-assay precision

Samples were assayed 48 times in multiple assays to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	149.3	35.1	10.0
SD	5.7	2.3	0.9
%CV	3.8	6.7	9.0

SD = Standard Deviation: CV = Coefficient of Variation

Linearity of dilution

Mouse serum and tissue culture medium containing 10% fetal bovine serum were spiked with Ms IFN-γ 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	Cell Culture			Serum		
	Measured (pg/mL)	Expected (pg/mL)	% Expected	Measured (pg/mL)	Expected (pg/mL)	% Expected
1/4	329.5	392.8	84	49.9	61.0	82
1/8	197.7	196.4	101	29.0	30.5	95
1/16	101.6	98.2	103	15.8	15.3	104
1/32	53.4	49.1	109	9.1	7.6	119
1/64	16.3	24.5	118	3.8	3.8	100

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

DISCLAIMER: LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) DISCLAIM ALL WARRANTIES WITH RESPECT TO THIS DOCUMENT, EXPRESSED OR IMPLIED, INCLUDING BUT NOT LIMITED TO THOSE OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, OR NON-INFRINGEMENT. TO THE EXTENT ALLOWED BY LAW, IN NO EVENT SHALL LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) BE LIABLE, WHETHER IN CONTRACT, TORT, WARRANTY, OR UNDER ANY STATUTE OR ON ANY OTHER BASIS FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING BUT NOT LIMITED TO THE USE THEREOF.

© 2015 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified.

invitrogen

Mouse IFN-γ (improved) ELISA Kit

Catalog nos. KMC4021 — **Quantity:** 96 tests
KMC4022 — 192 tests
KMC4021C — 480 tests

Pub. No. MAN0004158 **Rev** 2.0

Description

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

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	KMC4021 96 tests	KMC4022 192 tests	KMC4021C 480 tests
Mouse IFN-γ Antibody Coated Wells. 96 well plate.	1 plate	2 plates	5 plates
Mouse IFN-γ Biotin Conjugate. Contains 0.1% sodium azide.	11 mL	2 × 11 mL	5 × 11 mL
Mouse IFN-γ Standard, recombinant Mouse IFN-γ. Contains 0.1% sodium azide. Refer to vial label for quantity and reconstitution volume.	2 vials	4 vials	10 vials
Wash Buffer Concentrate (25X).	100 mL	100 mL	2 × 100 mL
Standard Diluent Buffer. Contains 0.1% sodium azide.	25 mL	2 × 25 mL	5 × 25 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	3 × 25 mL
Stabilized Chromogen, Tetramethylbenzidine (TMB).	25 mL	25 mL	3 × 25 mL
Stop Solution.	25 mL	25 mL	3 × 25 mL
Adhesive Plate Covers.	4	8	20

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.

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.

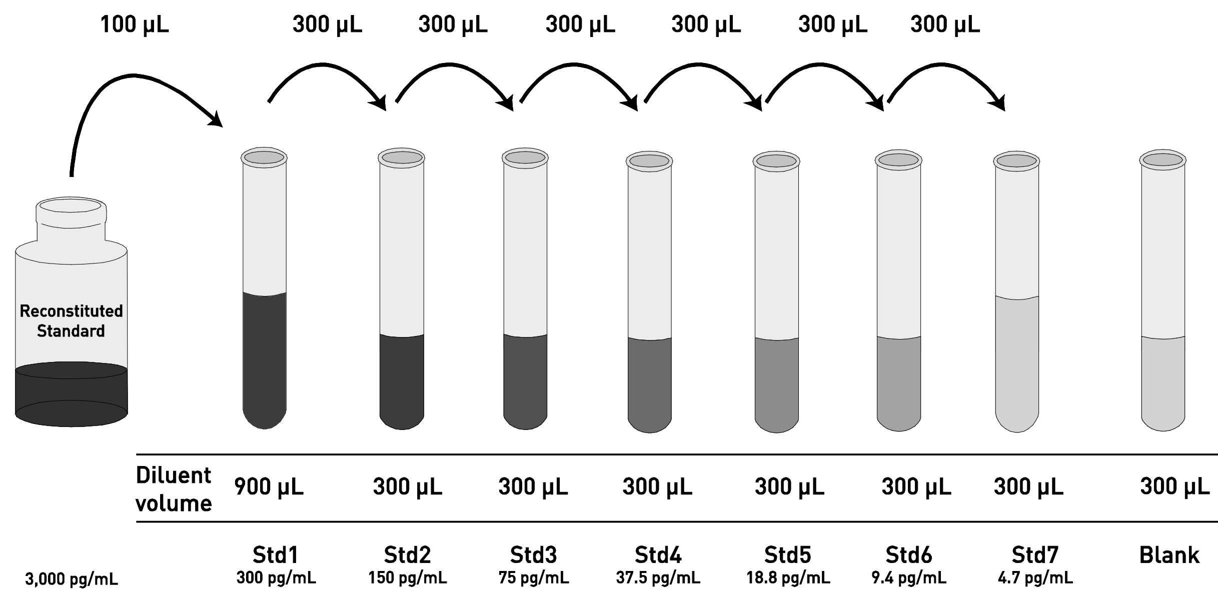
Dilute samples

- Dilute **serum**, **plasma**, and **tissue culture samples** 4-fold in Standard Diluent Buffer. Alternatively, samples may be diluted directly in the microtiter well by adding 75 µL of Standard Diluent Buffer to each well, followed by 25 µL of serum, plasma or tissue culture sample.
- 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 IFN-γ Standard to 3,000 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 3,000 pg/mL Ms IFN-γ.
   Use the standard within 15 minutes of reconstitution.
2. Add 100 µL Reconstituted Standard to one tube containing 900 µL Standard Diluent Buffer and mix. Label as 300 pg/mL Ms IFN-γ.
3. Add 300 µL Standard Diluent Buffer to each of 6 tubes labeled as follows: 150; 75; 37.5; 18.8; 9.4; and 4.7 pg/mL Ms IFN-γ.
4. Make serial dilutions of the standard as described below in the dilution diagram. Mix thoroughly between steps.
5. Discard remaining reconstituted standard. Return Standard Diluent Buffer to the refrigerator.



Prepare 1X Streptavidin-HRP solution

Note: Prepare1X Streptavidin-HRP within 15 minutes of usage.

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

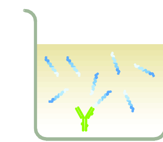
1. Allow Streptavidin-HRP (100X) to reach room temperature and mix gently.
2. For each 8-well strip used in the assay, pipet 10 µL Streptavidin-HRP (100X) solution, wipe the pipette tip with a clean absorbent paper to remove any excess solution, and dispense the solution into a tube containing 1 mL of 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 4 hours.**

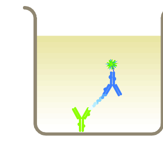
IMPORTANT! Perform a standard curve with each assay.

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.



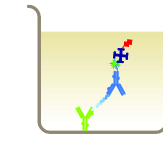
Bind antigen

1. Add 100 µL Standard Diluent Buffer to zero standard wells. Wells for chromogen blank should be left empty.
2. Add 100 µL of standards or diluted samples (see Dilute samples) to the appropriate wells.
3. Cover the plate with the plate cover and incubate for 2 hours at room temperature.
4. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.



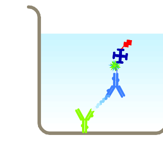
Add biotin conjugate

5. Add 100 µL Ms IFN-γ Biotin Conjugate solution into each well except the chromogen blanks.
6. Cover the plate with plate cover and incubate for 1 hour at room temperature.
7. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.



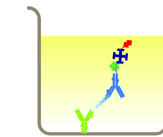
Add Streptavidin-HRP

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



Add chromogen

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



Add stop solution

13. 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 2 hours 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 0-300 pg/mL Ms IFN-γ.

Standard Ms IFN-γ (pg/mL)	Optical Density (450 nm)
300	3.41
150	2.26
75	1.23
37.5	0.60
18.8	0.31
9.4	0.17
4.7	0.09
0	0.02

Specificity

Buffered solutions of a panel of substances at 10,000 pg/mL were assayed with the Ms IFN-γ kit. The following substances were tested and found to have no cross-reactivity: **Human** IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-15, IL-17, IFN-α, IFN-γ, TNF-α, GM-CSF, MIP-1α, MIP-1β, IP-10, MIG, Eotaxin, RANTES, and MCP-1; **rat** IL-1β, IL-2, IL-4, IL-10, IL-13, MCP-1, and TNF-α; **mouse** IL-1β, IL-4, IL-5, IL-10, IL-17, GM-CSF, KC, TNF-α, and VEGF.

Sensitivity

The minimum detectable dose of Ms IFN-γ is <2 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.

Cross Reactivity

The cross-reactivity with recombinant rat IFN-γ was determined to be 1.7%.