IFN-γ

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. Monoclonal antibody specific for mouse IFN-γ has been pre-coated onto a microplate. Standards, Controls, and samples are pipetted into the wells and any mouse IFN-γ present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse IFN-γ is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the Stop Solution is added. The intensity of the color measured is in proportion to the amount of mouse IFN-γ bound in the initial step. The sample values are then read off the standard curve.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples with the appropriate Calibrator Diluent and repeat the assay.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

PRECAUTION

The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- For best results, pipette reagents and samples into the center of each well.
- It is recommended that the samples be pipetted within 15 minutes.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Stop Solution should be added to the plate in the same order as the Substrate Solution.

REAGENTS

Mouse IFN-γ Microplates (Part 890475) - Two 96 well microplates (12 strips of 8 wells) coated with monoclonal antibody specific for mouse IFN-γ.

Mouse IFN-γ Conjugate Concentrate (Part 890476) - 1 mL of a 23-fold concentrated solution containing polyclonal antibody against mouse IFN-γ conjugated to horseradish peroxidase, with preservatives.

Type 7 Conjugate Diluent (Part 895311) - 23 mL of diluent for diluting the conjugate concentrate, with preservatives.

Mouse IFN-γ Standard (Part 890477) - 3 vials (6 ng/vial) of recombinant mouse IFN-γ in a buffered protein base with preservatives, lyophilized.

Mouse IFN-γ Control (Part 890478) - 3 vials of mouse IFN-γ in a buffered protein base with preservatives, lyophilized. The concentration range of mouse IFN-γ after reconstitution is shown on the vial label. The assay value of the Control should be within the range specified on the Control label.

Assay Diluent RD1-21 (Part 895215) - 12.5 mL of a buffered protein solution, with preservatives.

Calibrator Diluent RD5Y (Part 895201) - 21 mL of a buffered protein solution, with preservatives. *For cell culture supernate samples.*

Calibrator Diluent RD6-12 (Part 895214) - 21 mL of a buffered protein solution, with preservatives. *For serum samples.*

Wash Buffer Concentrate (Part 895024) - 50 mL of a 25-fold concentrated solution of a buffered surfactant, with preservative.

Color Reagent A (Part 895000) - 12.5 mL of stabilized hydrogen peroxide.

Color Reagent B (Part 895001) - 12.5 mL of stabilized chromogen (tetramethylbenzidine).

Stop Solution (Part 895174) - 23 mL of a diluted hydrochloric acid solution.

Plate Covers (Part 640197) - 8 adhesive plate sealers.

STORAGE

Unopened Kit	Store at 2 - 8° C. Do not use beyond kit expiration date.	
Opened/ Reconstituted Reagents	Diluted mouse IFN-γ Conjugate	May be stored for up to 1 week at 2 - 8° C.*
	Diluted Wash Buffer	May be stored for up to 1 month at 2 - 8° C.*
	Stop Solution	
	Calibrator Diluent RD5Y	
	Calibrator Diluent RD6-12	
	Assay Diluent RD1-21	
	Conjugate Concentrate	
	Conjugate Diluent	
	Unmixed Color Reagent A	
	Unmixed Color Reagent B	
	mIFN-γ Standard (3000 pg/mL)	Use a new Standard and Control for each assay.
	Mouse IFN-γ Control	
	Microplate Wells	Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2 - 8° C.*

*Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- 1000 mL graduated cylinder.
- Deionized or distilled water.
- Multi-channel pipette, squirt bottle, manifold dispenser, or automated microplate washer.
- **Polypropylene tubes.**

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Serum - Allow blood samples to clot for 2 hours at room temperature or overnight at 2 - 8° C before centrifuging for 20 minutes at approximately 2000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Note: *Grossly hemolyzed or lipemic samples may not be suitable for measurement of mouse IFN-γ with this assay.*

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Mouse IFN-γ Kit Control - Reconstitute the Kit Control with 1.0 mL deionized or distilled water. Assay the Control undiluted.

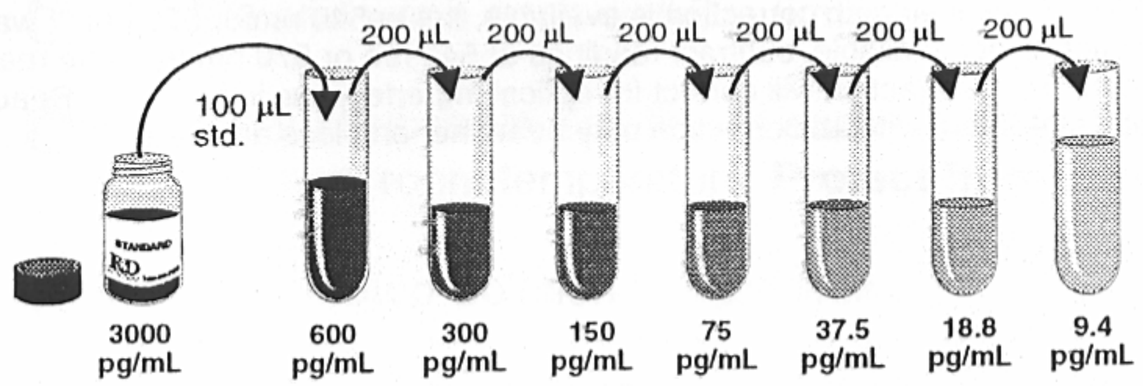
Mouse IFN-γ Conjugate - To prepare enough conjugate for one plate, add 0.5 mL of Conjugate Concentrate to 11.0 mL Conjugate Diluent. Use a sterile container, and protect from light.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. To prepare enough wash buffer for one plate, add 25 mL Wash Buffer Concentrate into deionized or distilled water to prepare 625 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 µL of the resultant mixture is required per well.

Mouse IFN-γ Standard - Reconstitute the mouse IFN-γ Standard with 2.0 mL of Calibrator Diluent RD5Y (*for cell culture supernate samples*) or Calibrator Diluent RD6-12 (*for serum samples*). Do not substitute other diluents. This reconstitution produces a stock solution of 3000 pg/mL. Allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Use polypropylene tubes. Pipette 400 µL of the appropriate Calibrator Diluent (*Calibrator Diluent RD5Y for cell culture supernate samples or Calibrator Diluent RD6-12 for serum samples*) into the 600 pg/mL tube. Pipette 200 µL of the appropriate Calibrator Diluent in the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 600 pg/mL standard serves as the high standard. The appropriate Calibrator Diluent serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples, standards and controls be assayed in duplicate.

1. Prepare reagents, samples and standards as directed by the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal.
3. Add 50 µL of Assay Diluent RD1-21 to each well.
4. Add 50 µL of Standard, Control or sample per well. Mix by gently tapping the plate frame for 1 minute. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature. Plate layouts are provided as a record of samples and standards assayed.
5. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of mouse IFN-γ Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
9. Add 100 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

PROCEDURE SUMMARY AND CHECKLIST

1. ☐ Bring all reagents to room temperature.
☐ Prepare reagents and samples as instructed.
☐ Return unused components to storage temperature as indicated in the instructions.

2. ☐ Add 50 µL Assay Diluent to the center of each well.

3. ☐ Add 50 µL Standard, Control, or sample to the center of each well.
☐ Tap plate gently for one minute.
☐ Cover the plate and incubate for 2 hours at room temperature.

4. ☐ Aspirate and wash each well five times.

5. ☐ Add 100 µL Conjugate to each well.
☐ Cover the plate and incubate 2 hours at room temperature.

6. ☐ Aspirate and wash each well five times.

7. ☐ Add 100 µL Substrate Solution to each well. Incubate 30 minutes at room temperature. **Protect from light.**

8. ☐ Add 100 µL Stop Solution to each well.

9. ☐ Read Optical Density at 450 nm (correction wavelength set at 540 nm or 570 nm).

CALCULATION OF RESULTS

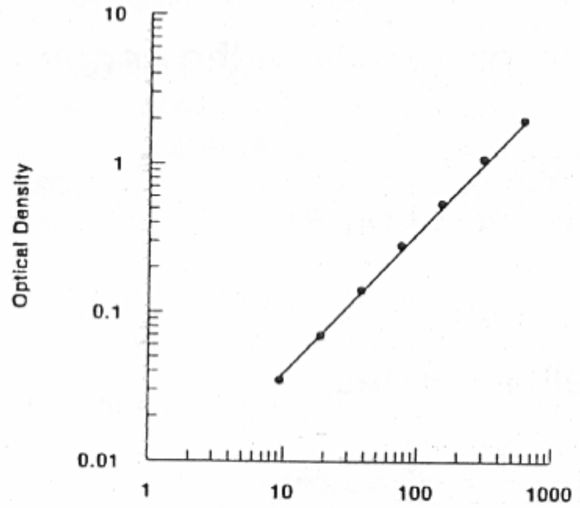
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density.

Plot the optical density for the standards versus the concentration of the standards and draw the best curve. The data can be linearized by using log/log paper and regression analysis may be applied to the log transformation.

TYPICAL DATA

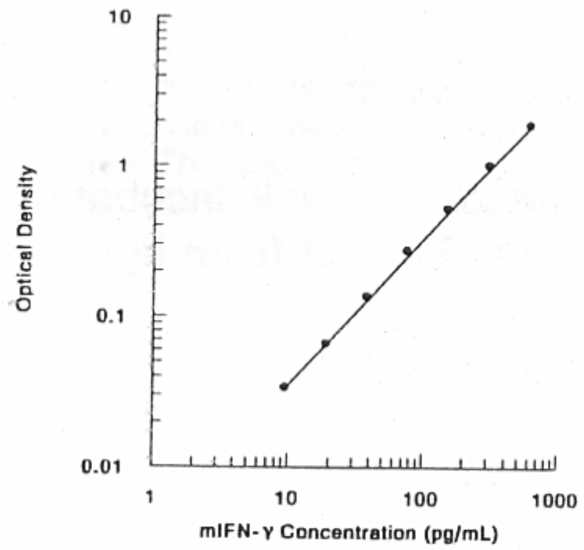
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Calibrator Diluent RD5Y



(pg/mL)	O.D.	Average	Corrected
0	0.042 0.043	0.042	—
9.4	0.077 0.077	0.077	0.035
18.8	0.113 0.112	0.112	0.070
37.5	0.183 0.186	0.184	0.142
75	0.327 0.329	0.328	0.286
150	0.580 0.594	0.587	0.545
300	1.130 1.124	1.127	1.085
600	2.020 2.042	2.031	1.989

Calibrator Diluent RD6-12



(pg/mL)	O.D.	Average	Corrected
0	0.035 0.037	0.036	—
9.4	0.069 0.071	0.070	0.034
18.8	0.099 0.107	0.103	0.067
37.5	0.170 0.179	0.174	0.138
75	0.309 0.318	0.314	0.278
150	0.551 0.576	0.564	0.528
300	1.053 1.081	1.067	1.031
600	1.950 2.005	1.978	1.942

PRECISION

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in twenty assays to assess inter-assay precision.

Cell Culture Supernate Assay

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	44.8	156	518	43.3	144	509
Standard deviation	2.2	5.0	14.2	3.6	14.7	48.6
CV (%)	4.9	3.2	2.7	8.3	10.2	9.5

Serum Assay

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	45.1	143	528	42.8	139	518
Standard deviation	2.1	4.4	11.8	3.6	13.6	46.5
CV (%)	4.7	3.1	2.2	8.4	9.8	9.0