MicroFluere® Human Interferon Gamma (IFNG) ELISA Kit

Intended Use

This kit is developed for quantitative measurement of natural and recombinant human Interferon Gamma (IFNG). This kit contains sufficient materials (except for not provided materials) to run five MicroFluere® well plates. This entire instruction must be read before using this product. In addition, watching the following video is highly recommended.

https://www.optobio.com/wpcontent/uploads/2021/04/Drainage-device-1.mp4.

This kit is intended for research use only (RUO).

Principle of the Assay

This assay is based on the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human Interferon Gamma (IFNG) has been pre-coated inside the microfluidic channels of the MicroFluere® well plate. Standards and samples are pipetted into the wells and any IFNG present is bound by the immobilized antibody. A horseradish peroxidase conjugated rabbit anti- Interferon Gamma monoclonal antibody is then added, producing an antibody-antigen-antibody "sandwich". The wells are washed and then QuantaRed™ substrate solution is loaded, which produces fluorescence in proportion to the amount of Human Interferon Gamma (IFNG) present in the sample. Finally, acquire the fluorescence intensity (~550 nm excitation and 605 nm emission wavelengths) at desired substrate incubation time point.

Materials Provided

1. MicroFluere® well plate coated with a mouse anti-Human Interferon Gamma (IFNG antibody, rabbit monoclonal)
2. Standard- Each vial contains 40 ng* of recombinant Interferon Gamma (IFNG)
3. Detection Antibody – 0.2 mg/mL* of mouse anti-Human Interferon Gamma, IFNG monoclonal antibody conjugated to horseradish-peroxidase (HRP) (in PBS, 50% HRP-Protector)
4. Dilution Buffer (A) for sample and standard
5. Dilution Buffer (B) for detection antibody
6. 100 absorbent pads

*Concentration can be varied lot to lot. Please read certificate of analysis (C of A).

Materials and Equipment Required (Not Provided)

1. MicroFluere® specific Drainage Device
   The device can be used over 500 plates. (The body of device is good for the lifetime and a replaceable latch of the device can be used over 500 plates.)
   https://www.optobio.com/product/microfluere-specific-drainage-device/
2. Substrate
   QuantaRed™ Enhanced Chemifluorescent HRP Substrate Kit (Catalog number: 15159)
   1 kit is good enough for 50 MicroFluere® well plates.
   https://www.thermofisher.com/order/catalog/product/15159#/15159

3. Washing buffer (1X PBS + 0.05% Tween20)
   Quantikine ELISA Wash Buffer 1 (WA12)
   https://www.rndsystems.com/products/quantikine-elisa-wash-buffer-1_wa126
   or
   Invitrogen™ eBioscience™ ELISA Wash Buffer, 1L packet (50-184-79)
   https://www.fishersci.com/shop/products/elisa-wash-buffer/5018479)

   or any other sources

4. 1X PBS
   PBS, Phosphate Buffered Saline, 10X Powder, pH 7.4, Fisher BioReagents
   https://www.fishersci.com/shop/products/pbs-10x-powder-concentrate-white-granular-powder-fisher-bioreagents/BP6651

   or any other sources

5. Fluorescence Microplate Reader (~550 nm excitation and 605 nm emission wavelengths)

Storage

(1) MicroFluere® well plate, Detection Antibody, and Buffers: Store at 4℃ and protect it from prolonged exposure to light for up to 6 months from date of receipt. DO NOT FREEZE!
(2) Standard: Store lyophilized standard at -20℃ to -80℃ for up to 6 months from the date of receipt. Aliquot and store the reconstituted standard at -80℃ for up to 1 month. Avoid repeated freeze-thaw cycles.

ELISA Protocol

Reagent Preparation

Bring all reagents and MicroFluere® to room temperature before use. Working dilutions should be prepared and used immediately.

Recombinant Human IFNG Standard: Reconstitute with 0.2 mL Standard Dilution Buffer (A). After reconstitution, store at -20C to -80C in a manual defrost freezer. A standard curve using 2-fold serial dilutions in Standard Dilution Buffer, and a high standard of 12 ng/mL. Recommend using the standard immediately (within 15 minutes).

HRP conjugated Anti-Human IFGN Detection Antibody: Dilute to working concentration of 350 ng/mL in Dilution Buffer (B) provided in the kit before use.

Assay Procedure

1. Make standards start with the highest concentration and serial dilutions; Dispense 20 μL in each well; Incubate 15 minutes and then drain the solution using the drainage device

In this step, samples can be loaded together with the calibration standards.

2. Make a working solution of HRP conjugated detection antibody; Dispense 15 μL in each well; Incubate 15 minutes and then drain the solution using the drainage device.

3. Wash with washing buffer (i.e., dispense 20 μL in each well and then drain the solution using the drainage device), repeat 2 times

4. Wash with PBS buffer (i.e., dispense 20 μL in each well and then drain the solution using the drainage device)

5. Make working solution of substrate (50:50:1 peroxide: enhancer: ADHP concentrate QuantaRed); Dispense 13 μL/well and incubate covered 5-15 minutes. Do not use stop solution. Do not drain the substrate. The plate is ready to read with a fluorescence microplate reader.

6. Read with a Fluorescence microplate reader (~550 nm excitation and 605 nm emission wavelengths)

Summary Table of MicroFluere® ELISA Protocol

Step	Concentration	Buffer	Volume (μL)	Incubation (min)
Standard/Sample	5.9 - 12000 pg/mL	Dilution Buffer (A)	20	15
HRP conjugated Detection Antibody	350 ng/ml	Dilution Buffer (B)	15	15
Wash 3 times	--	1x washing buffer	20	--
Wash 1 time	--	1x PBS	20	--
Substrate	--	--	13	5-15

Note

Reagent preparations note: When mixing antibody/standard for working solutions DO NOT vortex them. Use only pipette gently up and down to avoid bubble formation. Vortex is okay for Substrate. (Please see more detail in liquid handling tips section.)

Drainage device note: Insert a new absorbent pad and closes the lid. Wait for ~30 seconds before pressing the pump three to five times. Wait for ~20 seconds, then remove the plate. (Please see more detail in the Drainage Device video.)

Calculation of Results

Average the duplicate readings for each standard, control, and sample. Create a standard curve with a four-parameter logistic (4-PL) curve-fit. Unknown concentrations of the sample can be calculated using fluorescence intensity read out with the curve fit. If samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor.

Typical Data

This standard curve is only for demonstration purposes. A standard curve should be generated for each assay.