EZElisa™ Human IFN-α/IFN alpha ELISA Kit

Cat #: D-AEK6010

Size: 48T / 96T

Storage: Stored at 4°C for 12 months, protected from light

Product Information

Detection range: 46.9 pg/mL-3,000 pg/mL

Sensitivity: 46.9 pg/mL

Specificity: EZElisa™ Human IFN-α ELISA Kit has high sensitivity and excellent specificity for detection of Human IFN-α. No significant cross-reactivity or interference between Human IFN-α and analogues was observed.

Applicable samples: Serum, Plasma, Cell culture supernatants

Assay Principle

The interferons (IFN)s are a family of cytokines with potent antiviral, antiproliferative and immunomodulatory properties. The protein encoded by IFN-α gene is produced by macrophages and has antiviral activity. EZElisa™ Human IFN-α ELISA Kit employs a double antibody sandwich method to quantitate Human IFN-α in samples. An antibody specific for Human IFN-α has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Human IFN-α present is bound by the immobilized antibody. After removing any unbound substances, a HRP-conjugated antibody specific for Human IFN-α is added to the wells. After washing, remove any unbound HRP-conjugated antibody reagent, adding HRP Substrate (TMB), TMB turns blue under the catalysis of HRP, and turns yellow after adding stop solution. Measure the OD value with a microplate reader at 450 nm wavelength. The Human IFN-α concentration is proportional to the OD450 nm value.

Materials Supplied and Storage Conditions

Kit components	Size（48T）	Size（96T）	Storage conditions
Human IFN-α Microplate	48 wells	96 wells	4°C
Human IFN-α Standard (lyophilized)	1	2	4°C
Sample Diluent (5×)	3.5 mL	7 mL	4°C
Assay Buffer (5×)	3.5 mL	7 mL	4°C
HRP-conjugated Human IFN-α Detect Antibody (100×)	60 µL	120 µL	4°C
HRP Substrate (TMB)	5 mL	10 mL	4°C, protected from light
Stop Solution	5 mL	10 mL	4°C
Wash Buffer (20×)	25 mL	50 mL	4°C
Plate Covers	1	2	RT

Materials Required but Not Supplied

·Microplate reader capable of measuring absorbance at 450 nm

·Multi channel pipette or automated microplate washer

·Incubator, refrigerated centrifuge

·Precision pipettes, disposable pipette tips

·Deionized water

Reagent Preparation

1×Sample Diluent: Sample Diluent (5×) equilibrate to room temperature and dilute with deionized water 1:5 to obtain the 1×Sample Diluent before use. Mix gently to avoid foaming. Store at 4°C. This solution is stable for 30 days. If your samples need to be diluted, 1×Sample Diluent is used for dilution of standard, serum and plasma samples.

1×Assay Buffer: Assay Buffer (5×) equilibrate to room temperature and dilute with deionized water 1:5 to obtain the 1×Assay buffer before use. Mix gently to avoid foaming. Store at 4°C. This solution is stable for 30 days. 1×Assay buffer is used for dilution of HRP-conjugated Human IFN-α Detect Antibody (100×).

Human IFN-α Standard: Reconstitute the Human IFN-α standard in 1 mL of 1×Sample Diluent for a concentration of 3,000 pg/mL. Allow the standard to sit for a minimum of 15 min with gentle shake prior to making dilutions.

1×HRP-conjugated Human IFN-α Detect Antibody: Mix well prior to making dilutions. Make a 1:100 dilution of the concentrated detect antibody solution with 1×Assay buffer in a clean plastic tube as needed according to the standards and samples. 1×HRP -conjugated Human IFN-α Detect Antibody should be used within 30 min.

HRP Substrate (TMB): Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C, protected from light.

Stop Solution: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

1×Wash Buffer: Equilibrate to room temperature and dilute with deionized water 1:20 to obtain the 1×Wash buffer before use. Mix gently to avoid foaming. Store at room temperature. Please note that 1×Wash buffer is stable for 30 days.

Standard Curve Setting: dilute 3,000 pg/mL standard with 1×Sample Diluent to 3,000, 1,500, 750, 375, 187.5， 93.75, 46.9 and 0 pg/mL of Human IFN-α standard just as below.

Num.	Volume of Standard	Volume of 1×Sample Diluent (μL)	The concentration of Standard （pg/mL）
Std.1	1,000 μL of 3000 pg/mL	0	3000
Std.2	500 μL of Std.1 (3000 pg/mL)	500	1500
Std.3	500 μL of Std.2 (1500 pg/mL)	500	750
Std.4	500 μL of Std.3 (750 pg/mL)	500	375
Std.5	500 μL of Std.4 (375 pg/mL)	500	187.5
Std.6	500 μL of Std.5 (187.5 pg/mL)	500	93.75
Std.7	500 μL of Std.6 (93.75 pg/mL)	500	46.9
Std.8	0	500	0

Note：Always prepare a fresh set of standards per use.

Sample Preparation

1.Cell culture supernatants: Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20°C. Avoid repeated freeze-thaw cycles.

2.Serum: Use a serum separator tube and allow samples to clot for 30 min at room temperature before centrifugation for 15 min at 1,000 g. Remove serum and assay immediately or aliquot and store samples at -20°C. Avoid repeated freeze-thaw cycles.

3.Plasma: Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 min at 1,000 g within 30 min of collection. Assay immediately or aliquot and store samples at -20°C. Avoid repeated freeze-thaw cycles.

Note: Do not use grossly hemolyzed or lipemic specimens. If samples are to be used within 24 h, they may be stored at 2 to 8°C. Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

Assay Procedure

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal. The strips used for testing are equilibrated to room temperature before use.

2. Add 100 µL of diluted standard or sample per well. It is recommended that all Standards and Samples be added in duplicate to the microplate. Cover with the plate cover provided. Incubate for 2 h at room temperature.

3. Remove liquid in each well and wash, repeating the process for a total of three washes. Wash by filling each well with 1×Wash Buffer (250 µL) using a multi channel pipette or automated microplate washer, and let it stand for 1-2 min, complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining 1×Wash Buffer by invert the plate and blot it against clean paper towels.

4. Add 100 µL of diluted 1×HRP-conjugated Human IFN-α detect antibody to each well. Cover with the plate cover provided. Incubate for 1 h at room temperature.

5. Repeat the wash process for five times as in step 3.

6. Add 100 µL of HRP Substrate (TMB) to each well. Cover the plate and incubate for 15 min at room temperature. Protect from light.

7. Add 50 µL of Stop solution to each well. Stop Solution should be added to the plate in the same order as TMB. The color in the wells should change from blue to yellow. If the color in the wells is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

8. Determine the optical density of each well within 30 min, using a microplate reader set to 450 nm.

Data Analysis

1.Average the duplicate readings for each standard and sample and subtract the average zero standard (Std.8) optical density (O.D.).

2.Drawing of standard curve: With the standard solution concentration as the x-axis and the mean absorbance for each standard as the y-axis, draw the standard curve. A computer software can be used to create a standard curve.

Note: If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Typical Data

Typical standard curve （R^2≥0.99）

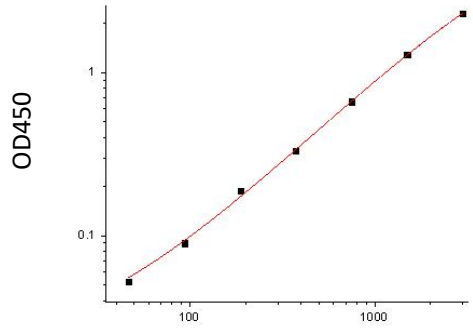

Fig.1.Standard Curve of Human IFN-α in 96-well plate assay, data provided for demonstration purposes only. A new standard Curve must be generated for each assay

Precautions

1.If Sample Diluent (5×) and Assay Buffer (5×) appears to turn yellow or a small amount of precipitation, etc., it is caused by the serum contained in the reagent. Please centrifuge to remove the precipitate, which will not affect normal use.

2.Do not mix or substitute reagents with those from other lots or sources.

3.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.

4.To ensure accurate results, proper adhesion of plate covers during incubation steps is necessary.

5.Stop Solution has certain Corrosive. Please take protective measures when operating.

FAQ

Problem	Cause	Suggested Solution
Poor standard curve	Inaccurate Pipetting.	Check pipettes
	Improper standard dilution.	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too short.	Ensure sufficient incubation times; increase to 2 or 3 h standard/ sample incubation
	Inadequate reagent volumes or improper dilution.	Check pipettes and ensure correct preparation.
	Incubation times with TMB too short.	Ensure sufficient incubation time until blue color develops prior addition of Stop solution.
High background /Large CV	Plate is insufficiently Washed.	Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed
	Contaminated Wash Buffer.	Make fresh Wash Buffer
Low sensitivity	Improper storage of the ELISA kit.	Store your reconstituted standards at -20°C (avoid repeated freeze-thaw cycles), all other assay components 4°C. Keep TMB Development Solution protected from light
	Stop solution.	Stop solution should be added to each well before measurement.

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.