Datasheet for ABIN5564594

IFNA2 ELISA Kit

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Overview

Quantity:	96 tests
Target:	IFNA2
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	31.25-2000 pg/mL
Minimum Detection Limit:	31.25 pg/mL
Application:	ELISA

Product Details

Purpose:	The AssayMax™ Interferon alpha-2b (IFN alpha-2b) ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human IFN alpha-2b in plasma, serum, and cell culture samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures human IFN alpha- 2b in approximately 4 hours. A polyclonal antibody specific for human IFN alpha-2b has been pre-coated onto a 96-well microplate with removable strips. IFN alpha-2b in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for human IFN alpha-2b, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.
Brand:	AssayMax™
Sample Type:	Cell Culture Cells, Plasma, Serum
Analytical Method:	Quantitative

Product Details

Detection Method:	Colorimetric
Components:	Human IFN alpha-2b Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human IFN alpha-2b. Sealing Tapes: Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay. Human IFN alpha-2b Standard: Human IFN alpha-2b in a buffered protein base (4000 pg, lyophilized, 2 vials). Biotinylated Human IFN alpha-2b Antibody (50x): A 50-fold concentrated biotinylated polyclonal antibody against human IFN alpha-2b (120 l). MIX Diluent Concentrate (10x): A 10-fold concentrated buffered protein base (30 ml). Wash Buffer Concentrate (20x): A 20-fold concentrated buffered surfactant (30 ml, 2 bottles). Streptavidin-Peroxidase Conjugate (SP Conjugate): A 100-fold concentrate (80 l). Chromogen Substrate: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml). Stop Solution: A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).
Material not included:	Microplate reader capable of measuring absorbance at 405 nm. Pipettes (1-20 µL, 20-200 µL, and multiple channel). Deionized or distilled reagent grade water Incubator (37 °C)

Target Details

Target:	IFNA2
Alternative Name:	Interferon alpha-2b (IFNA2 Products)
Background:	Interferon alpha-2b (IFN alpha-2b), also known as interferon alpha-A (LeIF A), and interferon alpha-2 (IFN alpha-2), belongs to the type I interferon family. The mature protein contains 165 amino acids with a molecular mass of 19 kDa (1). It exists in the crystal as a noncovalent dimer, which associates in a novel manner. Unlike other structurally characterized cytokines, zinc ion mediates extensive interactions in the dimer interface (2). It binds to interferon cell receptors type I and is encoded on chromosome 9. The heterodimeric alpha receptor consists of two subunits, IFNAR1 and IFNAR2, associating upon binding of interferon. The IFNAR2 subunit is the major ligand-binding component and can bind to IFN alpha-2b with high affinity. As a helical cytokine, IFN alpha-2b is produced by leukocytes in response to viral infections and has antiviral, antibacterial, antiproliferative, immunomodulatory, and cell growth regulatory activities (3-4).
Gene ID:	3440
UniProt:	P01563
Pathways:	JAK-STAT Signaling, Regulation of Leukocyte Mediated Immunity, Production of Molecular Mediator of Immune Response, Hepatitis C

Application Details

Assay Time:	5 h
Plate:	Pre-coated
Protocol:	• Step 1. Add 50 µL of Standard or Sample per well. Incubate 2 hours. • Step 2. Wash, then add 50 µL of Biotinylated Antibody per well. Incubate 1 hour. • Step 3. Wash, then add 50 µL of SP Conjugate per well. Incubate 30 minutes. • Step 4. Wash, then add 50 µL of Chromogen Substrate per well. Incubate 18 minutes. • Step 5. Add 50 µL of Stop Solution per well. Read at 450 nm immediately.
Reagent Preparation:	Freshly dilute all reagents and bring all reagents to room temperature before use. MIX Diluent Concentrate (10x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the MIX Diluent Concentrate 10-fold with reagent grade water. Store for up to 30 days at 2-8 °C. Human IFN alpha-2b Standard: Reconstitute the Human IFN alpha-2b Standard (4000 pg) with 1 mL of MIX Diluent to generate a 4000 pg/mL standard stock solution. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting from the standard stock solution (4000 pg/mL) 2-fold with MIX Diluent to produce 2000, 1000, 500, 250, 125, 62.5, and 31.25 pg/mL solutions. MIX Diluent serves as the zero standard (0 pg/mL). Aliquot remaining stock solution to limit repeated freeze-thaw cycles. This solution should be stored at -20 °C and used within 48 hours. 4 Standard Point Dilution [IFN alpha-2b] (pg/mL) P1 1 part Standard (4000 pg/mL) + 1 part MIX Diluent 2000 P2 1 part P1 + 1 part MIX Diluent 1000 P3 1 part P2 + 1 part MIX Diluent 500 P4 1 part P3 + 1 part MIX Diluent 250 P5 1 part P4 + 1 part MIX Diluent 125 P6 1 part P5 + 1 part MIX Diluent 62.5 P7 1 part P6 + 1 part MIX Diluent 31.25 P8 MIX Diluent 0.0 Biotinylated Human IFN alpha-2b Antibody (50x): Spin down the antibody briefly and dilute the desired amount of the antibody 50-fold with MIX Diluent. The undiluted antibody should be stored at -20 °C. Wash Buffer Concentrate (20x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 20-fold with reagent grade water. SP Conjugate (100x): Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 100-fold with MIX Diluent. The undiluted conjugate should be stored at -20 °C.
Sample Collection:	Plasma: Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x g for 10 minutes and collect plasma. The sample is suggested for use at 1x, however, user should determine optimal dilution factor depending on application needs. Samples can be stored at -20 °C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as an anticoagulant). Serum: Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x g for 10 minutes and remove serum. The sample is suggested for use at 1x, however, user should

	determine optimal dilution factor depending on application needs. Samples can be stored at -20 °C or below for up to 3 months. Avoid repeated freeze-thaw cycles. Cell Culture Supernatants: Centrifuge cell culture media at 3000 x g for 10 minutes at 4 °C to remove debris and collect supernatants. Samples can be stored at -20 °C or below. Avoid repeated freeze-thaw cycles.
Assay Procedure:	Prepare all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25 °C). Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator. Add 50 l of Human IFN alpha-2b Standard or sample to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition. Wash five times with 200 l of Wash Buffer manually. Invert the plate each time and decant the contents, hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 l of Wash Buffer and then invert the plate, decanting the contents, hit 4-5 times on absorbent material to completely remove the liquid. Add 50 l of Biotinylated Human IFN alpha-2b Antibody to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 1 hour. 5 Wash the microplate as described above. Add 50 l of Streptavidin-Peroxidase Conjugate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance. Wash the microplate as described above. Add 50 l of Chromogen Substrate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Incubate for 18 minutes or until the optimal blue color density develops. Add 50 l of Stop Solution to each well. The color will change from blue to yellow. Gently tap plate to ensure thorough mixing. Break any bubbles that may have formed. Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.
Calculation of Results:	• Calculate the mean value of the duplicate or triplicate readings for each standard and sample. • To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance (OD) on the y-axis. The best-fit line can be

Application Details

determined by regression analysis using log-log or four-parameter logistic curve-fit.

- Determine the unknown sample concentration from the standard curve and multiply the value by the dilution factor.

Restrictions:	For Research Use only

Handling

Handling Advice:	This product is for Research Use Only and is not intended for use in diagnostic procedures. 2 Prepare all reagents (diluent buffer, wash buffer, standard, biotinylated antibody, and SP conjugate) as instructed, prior to running the assay. Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this insert. However, the user should determine the optimal dilution factor. Spin down the SP conjugate vial and the biotinylated antibody vial before opening and using contents. The Stop Solution is an acidic solution. The kit should not be used beyond the expiration date.
Storage:	4 °C,-20 °C
Storage Comment:	Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date. Store Standard, SP Conjugate and Biotinylated Antibody at -20°C. Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C. Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator. Diluent (1x) may be stored for up to 30 days at 2-8°C. 3

Images

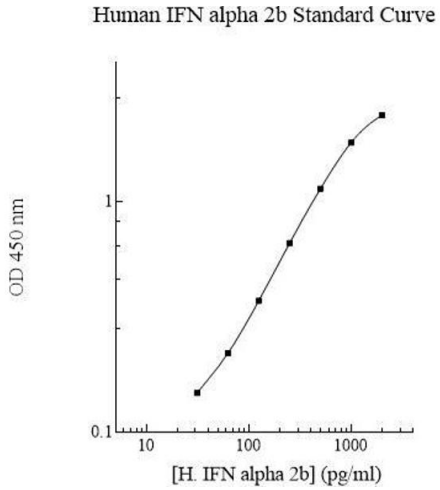

Image 1.