Datasheet for ABIN5564545

HSF1 ELISA Kit

Go to Product page

1 Image

Overview

Quantity:	96 tests
Target:	HSF1
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	0.625-20 ng/mL
Minimum Detection Limit:	0.625 ng/mL
Application:	ELISA

Product Details

Purpose:	The AssayMax™ Human HSF 1 ELISA (Enzyme-Linked Immunosorbent Assay) Kit is designed for detection of human HSF 1 in cell lysate and tissue extract samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures human HSF 1 in approximately 4 hours. A polyclonal antibody specific for human HSF 1 has been pre-coated onto a 96-well microplate with removable strips. HSF 1 in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for human HSF 1, which is recognized by a streptavidin- peroxidase (SP) 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, Tissue Lysate
Analytical Method:	Quantitative

Product Details

Detection Method:	Colorimetric
Components:	Human HSF 1 Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human HSF 1. Sealing Tapes: Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay. Human HSF 1 Standard: Human HSF 1 in a buffered protein base (12 ng, lyophilized, 2 vials). Biotinylated Human HSF 1 Antibody (40x): A 40-fold concentrated biotinylated polyclonal antibody against human HSF 1 (150 l). MIX Diluent Concentrate (10x): A 10-fold concentrated buffered protein base (30 ml). Standard Diluent (1x): A buffered protein base with stabilizer (2 ml). Wash Buffer Concentrate (20x): A 20-fold concentrated buffered surfactant (30 ml, 2 bottles). SP Conjugate (100x): A 100-fold concentrate (80 l). Chromogen Substrate (1x): A stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml). Stop Solution (1x): A 0.5 N hydrochloric acid solution 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:	HSF1
Alternative Name:	Heat Shock Factor Protein 1 (HSF 1) (HSF1 Products)
Background:	Heat shock factor protein 1 (HSF 1), also known as heat shock transcription factor 1 or HSTF 1, is a 529-amino acid transcription factor of 57 kDa (1). HSF 1 is present in unstressed cells as an inactive monomeric form and becomes activated by heat and other stress stimuli. HSF 1 is rapidly induced after temperature stress and binds heat shock promoter elements (HSE) that are present upstream of all the heat shock genes. Heat shock protein 90 (HSP90) inhibits HSF 1 activation. HSP90-containing HSF 1 complex is present in the unstressed cell and dissociates during stress (2). HSF 1 plays a crucial role in inducing heat shock proteins, which is required for thermotolerance. It is involved in oogenesis, spermatogenesis, placental development, and regulation of lifespan (3-4).
Gene ID:	3297
UniProt:	Q00613

Application Details

Assay Time:	4 h

Application Details

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 30 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. 4 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 to produce a 1x solution. Store for up to 30 days at 2-8 °C. Human HSF 1 Standard: Reconstitute the Human HSF 1 Standard (12 ng) with 0.3 mL of Standard Diluent to generate a 40 ng/mL standard stock solution. Allow the vial 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 (40 ng/mL) 2-fold with equal volume of MIX Diluent to produce 20, 10, 5, 2.5, 1.25, and 0.625 ng/mL solutions. MIX Diluent serves as the zero standard (0 ng/mL). Aliquot remaining stock solution to limit repeated freeze-thaw cycles. This solution should be stored at -20 °C and used within 48 hours. Standard Point Dilution [HSF 1] (ng/mL) P1 1 part Standard (40 ng/mL) + 1 part MIX Diluent 20 P2 1 part P1 + 1 part MIX Diluent 10 P3 1 part P2 + 1 part MIX Diluent 5 P4 1 part P3 + 1 part MIX Diluent 2.5 P5 1 part P4 + 1 part MIX Diluent 1.25 P6 1 part P5 + 1 part MIX Diluent 0.625 P7 MIX Diluent 0.0 Biotinylated Human HSF 1 Antibody (40x): Spin down the antibody briefly and dilute the desired amount of the antibody 40-fold with MIX Diluent to produce a 1x solution. 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 to produce a 1x solution. SP Conjugate (100x): Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 100-fold with MIX Diluent to produce a 1x solution. The undiluted conjugate should be stored at -20 °C.
Sample Collection:	Cell Lysate: Rinse cell with cold PBS and then scrape the cell into a tube with 5 mL of cold PBS and 0.5 M EDTA. Centrifuge suspension at 1500 rpm for 10 minutes at 4 °C and aspirate supernatant. Re-suspend pellet in ice-cold Lysis Buffer (10 mM Tris, pH 8.0, 130 mM NaCl, 1 % Triton X-100, protease inhibitor cocktail). For every 1 x 10 6 cells, add approximately 100 µL of ice-cold Lysis Buffer. Incubate on ice for 60 minutes. Centrifuge at 13000 rpm for 30 minutes at 4 °C and collect supernatant. Tissue Extract: Extract tissue samples with 0.1 M phosphate-buffered saline (pH 7.4) containing 1 % Triton X-100 and centrifuge at 14000 x g for 20 minutes. Collect the supernatant and measure the protein concentration. Freeze remaining

	extract at -20 °C or below.
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 5 securely to minimize exposure to water vapor and store in a vacuum desiccator. Add 50 l of Human HSF 1 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 HSF 1 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. Wash the microplate as described above. Add 50 l of SP 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 30 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 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. Prepare all reagents (diluent buffer, wash buffer, standard, biotinylated antibody, and SP conjugate) as instructed, prior to running the assay. 2 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, the biotinylated antibody vial, and the standard diluent 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), Standard Diluent (1x), 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. 3

Images

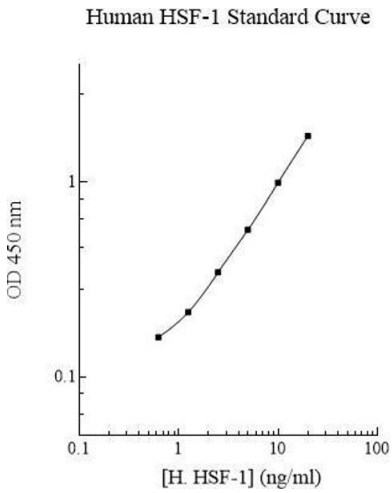

Image 1.