HSPD1 (Human) ELISA Kit

Catalog Number KA1843

96 assays

Version: 01

Intended for research use only

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Introduction and Background

A. Overview

Heat-shock protein of 60 kDa (HSPD1; Hsp60) is a mitochondrial chaperonin involved in folding, assembly, and transport of newly imported protein from cytoplasm into mitochondria in an ATP-mediated reaction (1-3). Human Hsp60 contains 573 amino acids, is related to the bacteria groEL protein, and located in the mitochondria and cytoplasm, the cell surface, the extracellular space, and the peripheral blood (4-5). Under dehydration conditions, the cytoplasmic Hsp60 is quickly imported into the mitochondria by cytoplasmic Hsp70 (6). Extracellular Hsp60 mediates apoptosis via Toll-like receptors in heart failure (7). Hsp60 plays a role in myelinogenesis and neurodegeneration and its defect can cause neurodegenerative pathologies (8). It has been suggested that Hsp60 links to diabetes, stress response, cancer and immunological disorders. Since it participates in cell signaling and key pathways, and is actively secreted by human tumor cells, Hsp60 could be used for cancer diagnosis and therapy (9).

B. Test Principle

The HSPD1 (Human) ELISA Kit is designed for detection of human HSPD1 in cell culture lysates, plasma, serum, and tissue samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures human HSPD1 in less than 5 hours. A polyclonal antibody specific for human HSPD1 has been pre-coated onto a 96-well microplate with removable strips. HSPD1 in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for HSPD1, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

C. Notice for Application of Kit

- ✓ **Prepare all reagents (working diluent buffer, wash buffer, standards, biotinylated-protein, 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 protocol. However, the user should determine the optimal dilution factor.**
- ✓ **Spin down the SP conjugate vial before opening and using contents.**
- ✓ This kit is for research use only.
- ✓ The kit should not be used beyond the expiration date.
- ✓ The Stop Solution is an acid solution.

Material and Method

A. List of component

- ✓ **Human Hsp60 Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human Hsp60.
- ✓ **Sealing Tapes:** Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.
- ✓ **Human Hsp60 Standard:** Human Hsp60 in a buffered protein base (40 ng, lyophilized, 2 bottles).
- ✓ **Biotinylated Hsp60 Antibody (70x):** A 70-fold concentrated biotinylated polyclonal antibody against Hsp60 (105 µL).
- ✓ **EIA Diluent Concentrate (10x):** A 10-fold concentrated buffered protein base (20 mL).
- ✓ **Wash Buffer Concentrate (20x):** A 20-fold concentrated buffered surfactant (30 mL, 2 bottles).
- ✓ **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrated (80 µL).
- ✓ **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).

B. Additional Required Materials But Not Provided

- ✓ Microplate reader capable of measuring absorbance at 450 nm
- ✓ Pipettes (1-20 µL, 20-200 µL, 200-1000 µL and multiple channel)
- ✓ Deionized or distilled reagent grade water

C. Sample Collection, Preparation and Storage

- ✓ **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 2000 xg for 10 minutes and assay. Store samples at -20℃ or below for up to 3 months. Avoid repeated freeze-thaw cycles. (EDTA or Heparin can also be used as anticoagulant.)
- ✓ **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2000 xg for 10 minutes. Collect the sample and assay. Store samples at -20℃ or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- ✓ **Cell Culture Lysates:** Place the cell culture dish in ice and wash the cells with ice-cold PBS. Drain the PBS, then add ice-cold lysis buffer (20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM Na_2EDTA, 1 mM EGTA, 1% Triton, 0.1mM PMSF, 1 µg/mL leupeptin, 1µg/mL aprotinin, and 1 µg/mL pepstatin.). Scrape adherent cells off the dish and then transfer the cell suspension into a pre-cooled microfuge tube. Maintain constant agitation for 30 minutes at 4℃. Centrifuge in a microcentrifuge at 4℃. Collect fresh cell lysates. Use undiluted samples or 1:2 diluted samples with EIA Diluent and assay. The undiluted samples can be stored at -200C or below. Tissue: Extract tissue samples with 50 mM phosphate-buffered saline (pH7.4) containing 1% Triton X-100 and centrifuge at 14000 xg for 20 min. Collect the supernatant and measure the protein concentration. Use undiluted samples or 1:2 diluted samples with EIA Diluent and assay. The undiluted samples can be stored at -20℃ or below.

D. Reagent Preparation

- ✓ Freshly dilute all reagents and bring all reagents to room temperature before use. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.
- ✓ **EIA Diluent Concentrate (10x):** Dilute the EIA Diluent 1:10 with reagent grade water. Store for up to 1 month at 2-8℃.
- ✓ **Standard Curve:** Reconstitute the 40 ng of Hsp60 Standard with 0.5 mL of EIA Diluent to generate a solution of 80 ng/mL. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the standard solution (80 ng/mL) 1:2 with EIA Diluent to produce 40, 20, 10, 5 and 2.5 ng/mL solutions. EIA Diluent serves as the zero standard (0 ng/mL). Any remaining solution should be discarded.

  Fresh standard should be reconstituted the day the assay is run.

Standard Point	Dilution	[Hsp60] (ng/mL)
P1	Standard (80 ng/mL)	80.0
P2	1 part P1 + 1 part EIA Diluent	40.0
P3	1 part P2 + 1 part EIA Diluent	20.0
P4	1 part P3 + 1 part EIA Diluent	10.0
P5	1 part P4 + 1 part EIA Diluent	5.00
P6	1 part P5 + 1 part EIA Diluent	2.50
P7	EIA Diluent	0.00

- ✓ **Biotin Hsp60 Antibody (70x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:70 with EIA Diluent. Any remaining solution should be frozen at -20℃.
- ✓ **Wash Buffer Concentrate (20x):** Dilute the Wash Buffer Concentrate 1:20 with reagent grade water.
- ✓ **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with EIA Diluent. Any remaining solution should be frozen at -20℃.

E. Stability and storage

- ✓ Store components of the kit at 2-8℃ or -20℃ upon arrival up to the expiration date.
- ✓ Store SP Conjugate and Biotinylated Antibody at -20℃.
- ✓ Store Microplate, Diluent Concentrate (10×), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8℃.
- ✓ Opened unused microplate wells may be returned to the foil pouch with the desiccant packs. Reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.
- ✓ Diluent (1×) may be stored for up to 1 month at 2-8℃.
- ✓ Store Standard at 2-8℃ before reconstituting with Diluent. Fresh standard should be reconstituted the day the assay is run.

F. Protocol

- ✓ Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-30℃).
- ✓ Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- ✓ Add 50 µL of **Hsp60 Standard** or sample per well. Cover wells with a sealing tape and incubate for two hours. Start the timer after the last sample addition.
- ✓ Wash five times with 200 µL of **Wash Buffer** manually. Invert the plate each time and decant the contents; hit it 4-5 times on absorbent paper towel to completely remove the liquid. If using a machine wash six times with 300 µL of **Wash Buffer** and then invert the plate, decant the contents; hit it 4-5 times on absorbent paper towel to completely remove the liquid.
- ✓ Add 50 µL of **Biotinylated Hsp60 Antibody** to each well and incubate for two hours.
- ✓ Wash the microplate as described above.
- ✓ Add 50 µL of **Streptavidin-Peroxidase Conjugate** to each well 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 µL of **Chromogen Substrate** per well and incubate for about 15 minutes or till the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- ✓ Add 50 µL of **Stop Solution** to each well. The color will change from blue to yellow.
- ✓ 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.

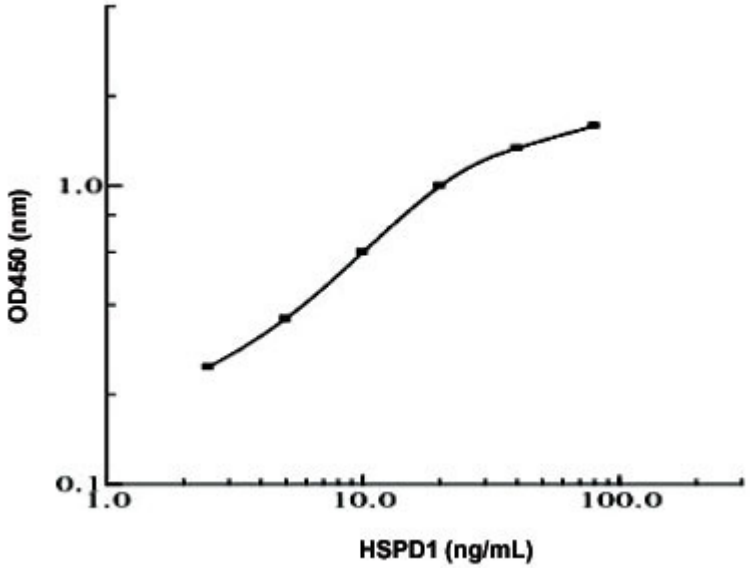
Result

A. Data analysis

- ✓ 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 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.

B. Standard Curve

- ✓ The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



Performance Characteristics

- ✓ The minimum detectable dose of Hsp60 is typically ~2 ng/mL.
- ✓ Intra-assay and inter-assay coefficients of variation were 4.8% and 7.2% respectively.

Recovery

Standard Added Value	2 – 20 ng/mL
Recovery %	86 – 106 %
Average Recovery %	97 %

References

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