Enzo Life Sciences

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> ImmunoSet® HSP27
high sensitivity (human), ELISA development set

Catalog # ADI-960-076
Reagents for 5 x 96-Well EIA Kits

This ImmunoSet contains the basic components for the development of an Hsp27 immunometric enzyme immunoassay (EIA). Each kit contains sufficient reagents for five 96-well plates.

This kit has been validated for use with cell lysates, serum, and EDTA plasma. Additional sample types will require validation by the user.

Visit www.enzolifesciences.com for tips and frequently asked questions.

Introduction

Hsp27 is one of the most common members of the highly conserved and ubiquitously expressed family of small heat shock proteins (sHsp), which also includes αB-crystallin[1]. It is characterized by a conserved C-terminal α-crystallin domain consisting of two anti-parallel β-sheets that promote oligomer formation required for its primary chaperone function as an inhibitor of irreversible protein aggregation[2]. Hsp27 oligomerization is modulated by post-translational phosphorylation of Hsp27 at three serine residues, Ser15, Ser78, and Ser82, by a variety of protein kinases[3,4]. Hsp27 inhibits actin polymerization by binding of unphosphorylated Hsp27 monomers to actin intermediate filaments[5], and is known to inhibit apoptosis[6-8].

Materials Provided

1. Hsp27 Capture Antibody

   One vial containing 312.5 µg lyophilized Hsp27 monoclonal antibody, Cat. #80-1945

2. Hsp27 Standard

   One vial containing 40 ng lyophilized recombinant Hsp27 protein, Cat. #80-1946

3. Hsp27 Detection Antibody

   One vial containing 25 µg lyophilized Hsp27 biotinylated polyclonal antibody, Cat. #80-1947

4. SA-HRP

   One vial containing 12.5 µg lyophilized streptavidin conjugated to horseradish peroxidase, Cat. #80-1896

Materials Needed but not Supplied

1. RIPA Cell Lysis Buffer, Cat. #80-1284, or similar
2. 96-well high-binding polystyrene microtiter plates, Cat. #80-1930, or similar
3. Precision pipets
4. Microplate reader capable of reading at 450 nm
5. Microplate shaker
6. Phosphate buffered saline (PBS)†
7. Tween®-20*†
8. Bovine Serum Albumin (BSA)†
9. 3,3′,5,5′ tetramethylbenzidine (TMB) solution, Cat. #80-1805 or similar†
10. 1N hydrochloric acid, such as Stop Solution 2, Cat. #80-1804†
11. Sucrose

†ImmunoSet Buffer Pack, Cat. #ADI-950-003

*Tween is a registered trademark of ICL Americas

Buffer Formulations

1. Coating Buffer

   10 mM sodium phosphate, 15 mM NaCl, pH 7.4

2. Blocking Buffer

   10 mM sodium phosphate, 15 mM NaCl, 1.0% BSA, 1.0% Sucrose, pH 7.4

3. Assay Buffer

   100 mM sodium phosphate, 150 mM NaCl, 1.0% BSA, 0.1% Tween-20, pH 7.4

4. Wash Buffer

   10 mM sodium phosphate, 15 mM NaCl, 0.1% Tween-20, pH 7.4

Plate Coating

1. Reconstitute Hsp27 Capture Antibody with 250 µL deionized water for a 250x stock. Use immediately, or make aliquots and freeze at -20°C for up to 3 months. For prolonged storage, aliquot and freeze at -80°C. Avoid repeated freeze/thaw cycles.
2. Dilute the stock 1:250 in Coating Buffer. Immediately dispense into 96-well microtiter plates using 100 µL of the diluted capture antibody per well. Seal the plate and incubate overnight at room temperature.
3. Aspirate each well to remove coating solution. Immediately add 200 µL Blocking Buffer per well. Seal the plate and incubate for at least 1 hour.
4. Aspirate each well to remove blocking solution. Plates may be used immediately or dried and stored with desiccant at 4°C.

Reagent Preparation

1. Recombinant Hsp27 Standard

   Reconstitute vial contents with 250 µL deionized water for a 160 ng/mL (50x) stock. Aliquot and store at -20°C for up to 3 months. For prolonged storage, aliquot and freeze at -80°C. Avoid repeated freeze/thaw cycles.

   The recommended standard curve range is 3.2 ng/mL to 0.1 ng/mL, using 2-fold serial dilutions in Assay Buffer. Do not store diluted standard.

2. Hsp27 Detection Antibody

   Reconstitute vial contents with 250 µL deionized water for a 250x stock. Store at 4°C for up to 3 months. For prolonged storage, aliquot and freeze at -20°C. Avoid repeated freeze/thaw cycles.

   Dilute the stock 1:250 in Assay Buffer for a working solution. Do not store diluted antibody.

3. SA-HRP

   Reconstitute vial contents with 250 µL deionized water for a 1000x stock. Store at 4°C for up to 3 months. For prolonged storage, aliquot and freeze at -20°C. Avoid repeated freeze/thaw cycles.

   Dilute the stock 1:1000 in Assay Buffer for a working solution. Do not store diluted conjugate.

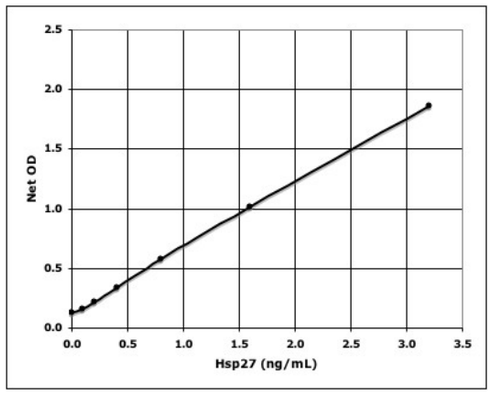
Assay Procedure

1. Pipet 100 µL of Assay Buffer into the control (0 ng/mL standard) wells.
2. Pipet 100 µL of standards and samples, prepared in Assay Buffer, to the bottom of the appropriate wells.
3. Seal the plate. Incubate for 1 hour on a plate shaker at room temperature.
4. Empty the contents of the wells and wash by adding 400 µL of Wash Buffer to every well. Repeat 3 more times for a total of 4 washes. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
5. Pipet 100 µL of the diluted detection antibody into each well, except the blank.
6. Seal the plate. Incubate for 1 hour on a plate shaker at room temperature.
7. Wash as above (Step 4).
8. Add 100 µL of the diluted conjugate to each well except the blank.
9. Seal the plate. Incubate for 30 minutes on a plate shaker at room temperature.
10. Wash as above (Step 4).
11. Pipet 100 µL of TMB solution into each well.
12. Seal the plate. Incubate for 30 minutes on a plate shaker at room temperature.
13. Pipet 100 µL 1N HCl into each well.
14. After blanking the plate reader against the substrate, read optical density at 450 nm. If the plate reader is not capable of adjusting for the blank, manually subtract the mean OD of the substrate blank from all readings.

Assay Performance

Typical Data

The results shown below are for illustration only and should not be used to interpret results from another assay.



Sensitivity

The sensitivity, or limit of detection, of this assay is 0.097 ng/mL. It was determined by interpolation at 2 standard deviations above the mean signal at background, using data from 9 standard curves.

Specificity

This assay detects Hsp27 in cell lysates, serum, and EDTA plasma of human origin. There is no cross reactivity observed with human Cpn10, Hsp32 and Hsp40, nor mouse Hsp25, nor bovine alphaB Crystallin.

Dilutional Linearity

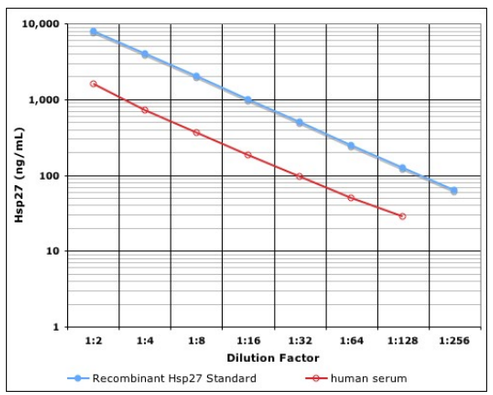
To determine possible interference from the sample matrix, the indicated sample types were serially diluted into assay buffer. The concentrations of Hsp27 were measured in the assay, and the results were analyzed to determine the range over which a linear response was obtained. These data may be used as a guideline to determine minimal recommended dilution (MRD) for similar samples.

Dilution Factor	HeLa CL	S	EDTA P
1:4	---	109%	---
1:8	---	98%	108%
1:16	---	97%	101%
1:32	---	100%	99%
1:64	112%	---	100%
1:128	111%	---	---
1:256	100%	---	---
1:512	100%	---	---

CL: Cell Lysate, **S:** Serum, **P:** Plasma

Parallelism

Dose-response curves from human serum diluted into assay buffer (using the MRD) were compared to the recombinant Hsp27 standard curve. Parallelism indicates antibody-binding characteristics of the native and standard proteins are similar, allowing accurate determination of analyte.



Calculation of Results

Several options are available for the calculation of the relative levels of Hsp27 in samples. We recommend that the data be handled by an immunoassay software package utilizing a 4 parameter logistic curve-fitting program. For accuracy, please ensure that sample values fall within the standard range.

Accessory Reagent List		
Reagent	Quantity	Cat. #
ImmunoSet® Buffer Pack	1 each of the following products: 80-1927, 80-1928, 80-1929, 80-1805, 80-1804	ADI-950-003
ImmunoSet® Plate Pack	5 96-well clear microtiter plates & 5 plate sealers	80-1930
PBS Concentrate	120 mL	80-1927
BSA Solution (10%)	50 mL	80-1928
Tween-20 Solution (10%)	30 mL	80-1929
RIPA Cell Lysis Buffer 2	100 mL	80-1284
Wash Buffer Concentrate	100 mL	80-1287
SA-HRP	12.5 µg/vial	80-1896

Storage

Store all components at 4˚C. See page 3 for storage of reconstituted material.

References

1. Kappe, G., *et al*. (2003) Cell Stress Chaperones **8**, 53-61.
2. Beck, F.X, *et al*. (2000) Am J Physiol Renal Physiol. **279**, F203-F215.
3. Landry, J., *et al*. (1992) J Biol Chem. **267**, 794-803.
4. Stokoe, D., *et al*. (1992) FEBS Lett. **313**, 307-313.
5. Butt, E., *et al*. (2001) J Biol Chem. **276**, 7108-7113.
6. Charette, S.J., *et al*. (2000) Mol Cell Biol. **20**, 7602-7612.
7. Rane, M.J., *et al*. (2003) J Biol Chem. **278**, 27828-27835.
8. Paul, C., *et al*. (2002) Mol Cell Biol. **22**, 816-834.

Tips & Troubleshooting

- ✓ If buffers other than those recommended are used in the assay, the end-user must determine the appropriate dilution and assay validation.
- ✓ Pipet the reagents to the sides of the wells to avoid possible contamination.
- ✓ Pre-rinse each pipet tip with reagent. Use fresh pipet tips for each sample, standard, and reagent.
- ✓ Insufficient washing or residual wash buffer in the wells may cause variation in assay results.
- ✓ Bring all reagents to room temperature for at least 30 minutes prior to opening.
- ✓ All standards, controls, and samples should be assayed in duplicate.

Limited Warranty

Catalog No. 25-0641 Last Revised: 01/31/2015