HSP60 ELISA Kit

Catalog number: EK7111

For detection of multiple analytes using one single assay.

This package insert must be read in its entirety before using this product.

For research use only. Not for use in diagnostic procedures.

BOSTER
antibody and ELISA experts

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HSP60 ELISA Kit

Catalog Number: EK7111

Size: 96wells/kit

Sample Type: Cell lysates, Serum, Tissue

Sensitivity: 1.73 ng/ml

Assay Range: 11-700 ng/ml

Storage: Store at 4°C.

Introduction

Boster's ELISA Kit is for the detection of Hsp60 in cell lysates, tissue extracts, and serum samples. Each kit contains sufficient components to quantitate the Hsp60 concentration in up to 40 samples, tested in duplicate.

Kit Components

Description	Quantity
Anti-Hsp60 Immunoassay Plate	1 Plate
5X Hsp60 Extraction Reagent	1 vial/10 ml
Recombinant Hsp60 Standard	1 vials/700ng
Standard and Sample Diluent	1 vial/ 50 ml
10X Wash Buffer Concentrate	1 vial/100 ml
Anti-Hsp60 Biotinylated Antibody Concentrate	1 vial/150 ul
Anti-Hsp60 Biotinylated Antibody Diluent	1 vial/ 13 ml
Streptavidin: HRP Concentrate	1 vial/150 ul
Streptavidin: HRP Diluent	1 vial/ 13 ml
TMB Substrate	1 vial/ 13 ml
Stop Solution	1 vial/ 13 ml

Materials Required, but Not Provided

1. Ultra pure water.

2. Additional reagents and materials for cell lysate and tissue extract preparation, including protease inhibitors.

3. Precision pipettors, with disposable plastic tips.

4. Polypropylene or polyethylene tubes to prepare samples – do not use polystyrene, polycarbonate or glass tubes.

5. A container to prepare 1X Wash Buffer.

6. A wash bottle or an automated 96-well plate washer.

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7. Disposable reagent reservoirs.

8. A standard microtiter plate reader for measuring absorbance at 450 nm.

9. Adhesive plate sealers.

Sample Dilution

1. Samples must first be diluted prior to testing.

2. Prepare at least 250 µL of sample in Standard and Sample Diluent. Mix samples well prior to analysis.

3. Suggested starting dilutions for samples:
o For cell and tissue lysates, prepare a 200 µg/mL total protein concentration dilution in Standard and Sample Diluent.
o When measuring Hsp60 in serum samples, use a 1:1 dilution in 50mM Acetic Acid (pH 4.67) and assay total 100 µL sample volume directly i.e. add 50 µl of 50 mM Acetic Acid to all standard and sample wells followed by 50 µL of appropriately diluted standards or samples to each well. Run each standard, sample, or blank in duplicate. Follow with the remaining procedure. Expect lower OD values, however, serum matrix effects are removed.

Note: If values for samples are not within the range of the standard curve, optimal sample dilutions need to be determined.

Standard Preparation

1. Reconstitute standard vial with 1 mL of Standard and Sample Diluent for a concentration of 700 ng/mL. Mix well.

2. Label seven (7) tubes, one for each additional standard curve point: 350 ng/mL, 175 ng/mL, 88 ng/mL, 44 ng/mL, 22 ng/mL, 11 ng/ml, and 0 ng/mL.

3. Pipet 250 µl of Standard and Sample Diluent into each tube.

4. Serial dilute the 700 ng/mL standard 1:1 with Standard and Sample Diluent. Perform dilution by mixing 250 µL of the previous standard with 250 µL of Standard and Sample Diluent. Continue until reach the standard value of 11 ng/ mL.

5. Use Standard and Sample Diluent only as the zero standard value.

1X Wash Buffer Preparation

Prepare 1X wash buffer by adding 100 ml of Wash Buffer Concentrate to 900 ml deionized or distilled water to prepare 1000 mL of Wash Buffer.

Biotinylated Antibody Working Solution Preparation

It is recommended to prepare this reagent immediately prior to use by diluting the Avidin-BiotinPeroxidase Complex 1:100 with Avidin-Biotin-Peroxidase Diluent. Prepare 100 µl by adding 1 µl of Avidin-Biotin-Peroxidase Complex to 99 µl of Avidin-Biotin-Peroxidase Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.

Streptavidin-HRP Working Solution Preparation

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It is recommended to prepare this reagent immediately prior to use by diluting Streptavidin-HRP Concentrate 1:100 with Streptavidin-HRP Diluent. Prepare 100 µl by adding 1 µl of Avidin-Biotin-Peroxidase Complex to 99 µl of Avidin-Biotin-Peroxidase Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.

Assay Overview

1. Prepare Standard and samples in Standard and Sample Diluent.

2. Add 100 µL of Standard or sample to appropriate wells.

3. Cover plate with Plate Sealer and incubate at 20°C-25°C for 1 hour.

4. Wash plate four times with 1X Wash Buffer.

5. Add 100 µL of Biotinylated Antibody Working Solution to each well.

6. Cover plate with Plate Sealer and incubate at 20°C-25°C for 1 hour.

7. Wash plate four times with 1X Wash Buffer.

8. Add 100 µL of Streptavidin-HRP Working Solution to each well.

9. Cover plate with Plate Sealer and incubate at room temperature for 30 minutes.

10. Wash plate four times with 1X Wash Buffer.

11. Add 100 µL of TMB Substrate to each well..

12. Develop the plate in the dark at room temperature for 30 minutes.

13. Stop reaction by adding 100 µL of Stop Solution to each well.

14. Measure absorbance on a plate reader at 450 nm.

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