> ImmunoSet® Grp75 ELISA development set

Catalog # ADI-960-143
Reagents for 5 x 96-Well EIA Kits

This kit has been validated for use with cell lysates. Additional sample types will require validation by the user.
Visit www.assaydesigns.com/FAQ for tips and frequently asked questions.

Introduction

Members of the Hsp70 family of heat shock proteins function as molecular chaperones[1], assisting in the folding of other proteins in various intracellular compartments[2]. Glucose-regulated 78 kDa protein (Grp75), also known as Mortalin or HspA9, resides in the mitochondrial matrix where it collaborates with Hsp60 in the re-folding of proteins translocated into this organelle[3]. Like its *E. coli* homolog DnaK[4], Grp75 possesses a cation-dependent ATPase activity[5] considered central to its function as a chaperone. Grp75 has been implicated in various cancers, cardiovascular disease, neurodegeneration, and aging[6].

References

1. Ellis, R.J. (1990) Semin Cell Biol. **1**, 1-9.
2. Gething, M.-J., *et al*. (1992) Nature **355**, 33-45.
3. Manning-Krieg, U.C., *et al*. (1991) EMBO J. **10**, 3273-3280.
4. Liberek, K., *et al*. (1991) J Biol Chem. **266**, 14491-14496.
5. Leustek, U.K., *et al*. (1989) PNAS USA **86**, 7805-7808.
6. Wadhwa, R. *et al*. (2002) Cell Stress Chaperones **7**, 309-316.

Materials Provided

1. Grp75 (Mortalin) Capture Antibody
   One vial containing 250 µg lyophilized Grp75 polyclonal antibody, Cat. #80-2081
2. Grp75 (Mortalin) Standard
   One vial containing 1.25 µg lyophilized recombinant human Grp75 protein, Cat. #80-2082
3. Grp75 (Mortalin) Detection Antibody
   One vial containing 6.25 µg each lyophilized Grp75 polyclonal antibody, Cat. #80-2083
4. SA-HRP
   One vial containing 12.5 µg lyophilized streptavidin conjugated to horseradish peroxidase, Cat. #80-1896

Materials Needed but not Supplied

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

†ImmunoSet Buffer Pack, Cat. #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
   25 mM Tris, pH 7.4, 10 mM KCl, 5 mM $MgCl_2$ 1.0% BSA, 0.1% Tween-20
4. Wash Buffer
   50 mM Tris, pH 7.5,100 mM NaCl, 0.05% Tween-20

Plate Coating

1. Reconstitute Grp75 (Mortalin) 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 -20°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 Grp75 (Mortalin) Standard

   Reconstitute vial contents with 250 µL deionized water for a 5.0 µg/mL (50x) stock. Aliquot unused portion and store at -20°C for up to 3 months. Avoid repeated freeze/thaw cycles.

   The recommended standard curve range is 100 to 3.125 ng/mL, using 2-fold serial dilutions in Assay Buffer. Do not store diluted standard.
2. Grp75 (Mortalin) 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.

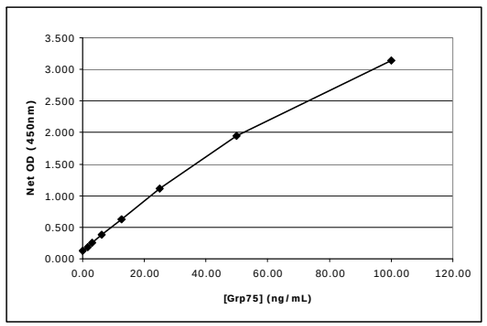
Assay Procedure

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 on a plate shaker for 1 hour 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 on a plate shaker for 1 hour 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 on a plate shaker for 30 minutes at room temperature.
10. Wash as above (Step 4).
11. Pipet 100 µL of TMB solution into each well.
12. Seal the plate. Incubate on a plate shaker for 30 minutes 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 0.762 ng/mL. It was determined by interpolation at 2 standard deviations above the mean signal at background, using data from 6 standard curves.

Specificity

This assay detects Grp75 in cell lysates from human, mouse, and rat. Cross-reactivity with related Hsp70 proteins is as listed below.

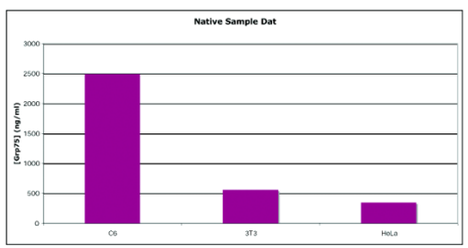
Cross Reactant	Species	% Cross-Reactivity
BiP	Hamster	<0.03%
DnaK	E. coli	<0.03%
Hsc70	Bovine	<0.03%
Hsp70	Human	<0.03%
Grp94	Canine	<0.078%
Hsp60	Human	<0.078%
Hsp90	Human	<0.078%

Dilutional Linearity

To determine possible interference from the sample matrix, the indicated sample types were serially diluted into the assay buffer. The concentrations of Grp75 were measured in the assay, and the results were analyzed to determine the range over which a linear response was obtained. From these data, the minimum required dilution (MRD) for cell lysates is 1:16. Similar recovery experiments should be performed to determine matrix effect for additional sample types.

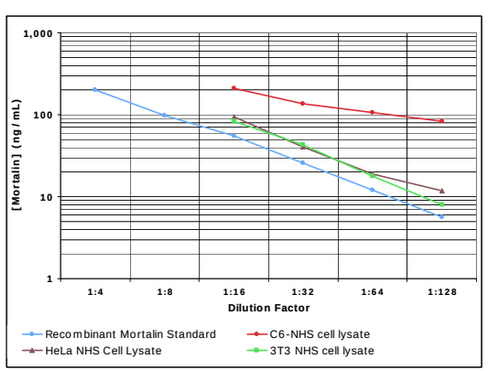
Native Sample Data

Concentrations of Grp75 were measured in lysates from either control or heat-shocked (HS) cells. Cell lysates were prepared using RIPA Cell Lysis Buffer 2, and were serially diluted into the assay buffer prior to the assay.



Parallelism

Dose-response curves from cell lysates diluted into assay buffer (using the MRD) were compared to the recombinant standard curve. A parallel response indicates the recombinant standard effectively mimics the native protein.



Calculation of Results

Several options are available for the calculation of the relative levels of Grp75 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 recommended 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	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
Wash Buffer Concentrate	100 mL	80-1287
SA-HRP	12.5 µg/vial	80-1896
RIPA Cell Lysis Buffer 2	100 mL	80-1284

*PBS concentrate (Cat. #80-1927) is suitable for use in coating and blocking buffers. The end-user must supply Tris-HCl for the assay buffer (see buffer formulations).

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

Enzo Life Sciences International, Inc. makes no warranty of any kind, expressed or implied, which extends beyond the description of the product in this brochure, except that the material will meet our specifications at the time of delivery. Enzo Life Sciences International, Inc. makes no guarantee of results and assumes no liability for injuries, damages or penalties resulting from product use, since the conditions of handling and use are beyond our control.

Catalog No. 25-0689 Last Revised: 01/27/2015