ELISA PRODUCT INFORMATION & MANUAL

gp96/HSP90B1/GRP94

NBP2-62146

Enzyme-linked Immunosorbent Assay for quantitative detection of Human, Mouse, Rat, Canine gp96/ HSP90B1/GRP94.
For research use only.
Not for diagnostic or therapeutic procedures.

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Novus kits are guaranteed for 6 months from date of receipt

> gp96/HSP90B1/GRP94 ELISA kit

Catalog # NBP2-62146
Reagents for 5 x 96-Well EIA Kits

This kit contains the basic components for the development of a Gp96/HSP90B1/GRP94 immunometric enzyme immunoassay (EIA). Each kit contains sufficient reagents for five 96-well plates.

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

Introduction

Glucose-regulated protein 94 (Grp94, gp96), an abundant resident endoplasmic reticulum (ER) lumenal stress protein, belongs to the Hsp90 family of molecular chaperones along with cytosolic Hsp90. Grp94 possess the C-terminal tetrapeptide Lys-Asp-Glu-Leu (KDEL), a sorting signal responsible for its retention in pre-Golgi compartments[1]. Stress conditions such as glucose starvation and heat shock which promote protein misfolding or unfolding increase Grp94 expression[2]. In addition to a homeostatic role in protein folding and assembly, Grp94 functions in the intracellular trafficking of peptides from the extracellular space to the MHC class I antigen processing pathway in antigen presenting cells[3,4].

References:

1. Peter, F., *et al*. (1992) J Biol Chem. **267**, 10631-10637.
2. Little, E., *et al*. (1994) Crit Rev Eukaryot Gene Expr. **4**, 1-18.
3. Nicchitta, C.V. (1998) Curr Opin Immunol. **10**, 103-109.
4. Srivastava, P.K., *et al*. (1998) Immunity **8**, 657-665.

Materials Provided

1. Gp96/HSP90B1/GRP94 Capture Antibody
   One vial containing 250 µg lyophilized Gp96/HSP90B1/GRP94 monoclonal antibody,
2. Recombinant Gp96/HSP90B1/GRP94 (Canine) Standard One vial containing 2.5 µg lyophilized recombinant Gp96/HSP90B1/GRP94 (Canine) protein
3. Gp96/HSP90B1/GRP94 Detection Antibody
   One vial containing 3.75 µg lyophilized, biotinylated Gp96/HSP90B1/GRP94 monoclonal antibody
4. SA-HRP
   One vial containing 12.5 µg lyophilized streptavidin conjugated to horseradish peroxidase

Materials Needed but not Supplied

1. Extraction Reagent, Cat. #80-1526 or similar
2. 96-well high-binding polystyrene microtiter plates
3. Precision pipets
4. Microplate reader capable of reading at 450 nm
5. Phosphate buffered saline (PBS) 6.

Tween®-20*†

7. Bovine Serum Albumin (BSA)
8. 3,3',5,5' tetramethylbenzidine (TMB) solution
9. 1N hydrochloric acid

*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, 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 Gp96/HSP90B1/GRP94 Capture Antibody with 250µL 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 3 hours.
4. Aspirate each well to remove blocking solution. Plates may be used immediately or dried and stored with desiccant at 4°C for up to 10 days.

Reagent Preparation

1. Recombinant Gp96/HSP90B1/GRP94 (Canine) Standard Reconstitute vial contents with 250 µL deionized water for a 10,000 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 200 ng/mL to 6.25 ng/mL, using 2-fold serial dilutions in Assay Buffer. Do not store diluted standard.
2. Gp96/HSP90B1/GRP94 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 500x 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 1:500 in Assay Buffer for a working solution. Do not store diluted conjugate.

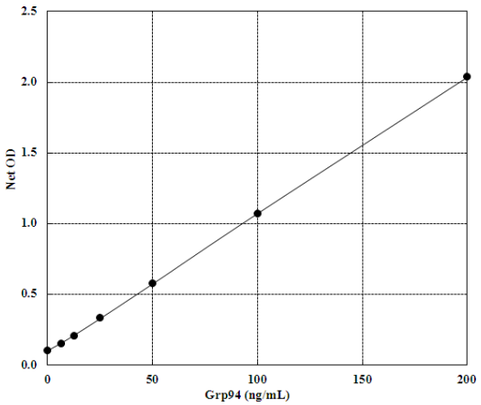
Assay Procedure

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 5 more times for a total of 6 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

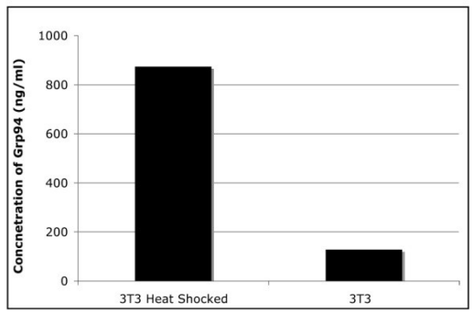
The sensitivity, or limit of detection, of this assay is 1.29 ng/mL. It was determined by interpolation at 2 standard deviations above the mean signal at background, using data from 8 standard curves.

Specificity

This assay detects Gp96/HSP90B1/GRP94 in cell lysates and microsomes. Reactivity with human, mouse, rat, and canine has been shown. Cross reactivity with human Calreticulin, PDI, Hsp90α, Hsp90β, Hsp70, hamster Grp78 and bovine Hsc70 is less than 0.035%.

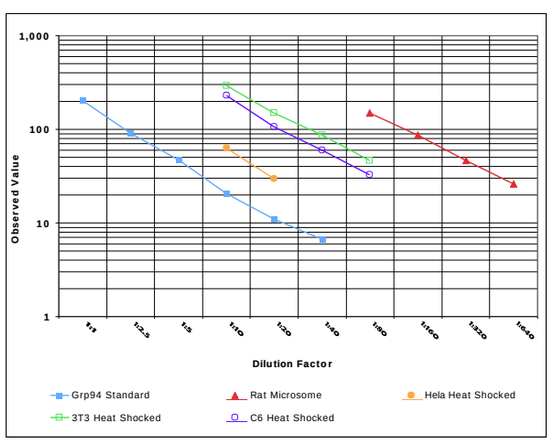
Native Sample Data

Concentrations of mouse Gp96/HSP90B1/GRP94 were measured in lysates from either control or heat-shocked 3T3 cells. Cell lysates were prepared using Extraction Reagent, and were serially diluted into assay buffer prior to the assay.



Parallelism

Dose-response curves from cell lysates and microsomes diluted into assay buffer were compared to the recombinant Canine Gp96/HSP90B1/GRP94 standard curve. Parallelism indicates that the antibody-binding characteristics of the native and standard proteins are similar, allowing accurate determination of the analyte.



Calculation of Results

Several options are available for the calculation of the relative levels of Gp96/HSP90B1/GRP94 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
Buffer Pack plus extra bottle of PBS concentrate	1 each of the following products: PBS concentrate, (120 ml) BSA solution (10%), (50 ml) Tween-20 solution (10%), (30 ml) TMB substrate, (50 ml) Stop solution 2, (50 ml)
Plate Pack	5 96-well clear microtiter plates & 5 plate sealers
PBS Concentrate	120 mL
BSA Solution (10%)	50 mL
Tween-20 Solution (10%)	30 mL
Extraction Reagent (5x)	10 mL
Wash Buffer Concentrate	100 mL
SA-HRP	12.5 µg/vial

Storage

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

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

Novus Biologicals 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. Novus Biologicals 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.