> ImmunoSet® PDI ELISA development set

Catalog # ADI-960-072
Reagents for 5 x 96-Well EIA Kits

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

This kit has been validated for use with cell lysates, cell culture supernatants, K_3EDTA plasma, and microsomes. Additional sample types will require validation by the user.

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

Introduction

The mammalian protein disulphide-isomerase (PDI) family encompasses several highly divergent proteins involved in the processing and maturation of secretory proteins in the ER by catalyzing the rearrangement of disulphide bonds[1,2]. PDI, an abundant protein of the ER (>400 µM), contains a carboxy-terminal retention signal sequence, KDEL, similar to that of BiP and Grp94[3]. The PDI proteins are characterized by the presence of one or more domains of ~95-110 amino acids related to the cytoplasmic protein thioredoxin.

References:

1. Ferrari, D.M. and Soling, H.D. (1999) Biochem J. **339**, 1-10.
2. Freedman, R.B. (1989) Cell **57**, 1069-1072.
3. Vaux, D., *et al*. (1990) Nature **345**, 495-502.

Materials Provided

1. PDI Capture Antibody
   One vial containing 219 µg lyophilized PDI monoclonal antibody, Cat. #80-1939
2. Recombinant PDI (human) Standard
   One vial containing 3.125 µg of lyophilized recombinant PDI (human) protein, Cat. #80-1940
3. PDI Detection Antibody
   One vial containing 25.625 µg lyophilized biotinylated PDI polyclonal antibody, Cat. #80-1941
4. SA-HRP

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

Materials Needed but not Supplied

1. Extraction Reagent, Cat. #ADI-80-1526, or similar
2. 96-well high-binding polystyrene microtiter plates, Cat. #ADI-80-1930, or similar
3. NaCl, reagent grade
4. $Na_2HPO_4 \bullet H_2O$ (m.w. 142), reagent grade
5. NaH_2PO_4 (anhydrous), reagent grade
6. Precision pipets
7. Microplate reader capable of reading at 450 nm
8. Phosphate buffered saline (PBS)†
9. Tween®-20*†
10. Bovine Serum Albumin (BSA)†
11. 3,3’,5,5’ tetramethylbenzidine (TMB) solution, Cat. #80-1805 or similar†
12. 1N hydrochloric acid, such as Stop Solution 2, Cat. #ADI-80-1804†

†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, pH 7.4
3. Assay Buffer

   100 mM sodium phosphate, 150 mM NaCl, 0.1% Tween-20, pH 7.4
4. Wash Buffer

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

Buffer Preparation

1. 100 mM sodium phosphate

   To prepare 1 L of 10 mM sodium phosphate, add 3.1 g of $NaH_2PO_4 \bullet H_2O$ and 10.9 g of Na_2HPO_4 (anhydrous) to distilled H_2O to make a volume of 1 L. The pH of the final solution will be 7.4.
2. 150 mM NaCl

   To prepare 1 L of 15 mM NaCl, dilute 8.76 g of NaCl to distilled H_2O to make a final volume of 1 L.
3. Coating Buffer
   Dilute appropriate amounts of 100 mM sodium phosphate and 150 mM NaCl to 10 mM and 15 mM, respectively. Add appropriate amounts of 10 mM sodium phosphate and 15 mM NaCl. Adjust pH to 7.4, if necessary.
4. Blocking Buffer
   Dilute appropriate amounts of 100 mM sodium phosphate and 150 mM NaCl to 10 mM and 15 mM, respectively. Add appropriate amounts of 10 mM sodium phosphate and 15 mM NaCl. Add 1.0% BSA. Adjust pH to 7.4, if necessary.
5. Assay Buffer
   Add appropriate amounts of 100 mM sodium phosphate and 150 mM NaCl. Add 0.1% Tween-20. Adjust pH to 7.4, if necessary.
6. Wash Buffer
   Dilute appropriate amounts of 100 mM sodium phosphate and 150 mM NaCl to 10 mM and 15 mM, respectively. Add appropriate amounts of 10 mM sodium phosphate and 15 mM NaCl. Add 0.1% Tween-20. Adjust pH to 7.4, if necessary.

Plate Coating

1. Reconstitute PDI 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 at room temperature.
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 PDI (human) Standard

   Reconstitute vial contents with 250 µL deionized water for a 12,500 ng/mL (50x) stock. Store at -20°C for up to 3 months. For prolonged storage, aliquot and freeze at -20°C. Avoid repeated freeze/thaw cycles.

   The recommended standard curve range is 250 ng/mL to 7.8 ng/mL, using 2-fold serial dilutions in Assay Buffer. Do not store diluted standard.
2. PDI 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 600x 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.

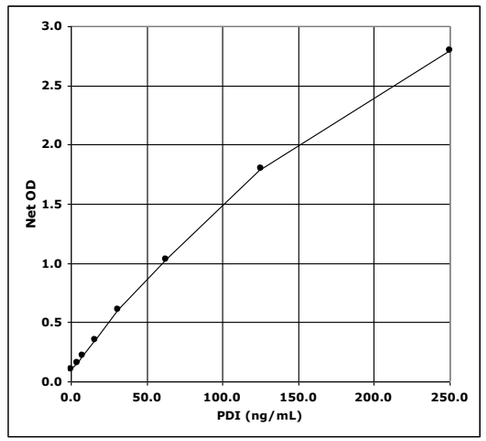
Assay Procedure

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 3.93 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 PDI in cell lysates, cell culture supernatant, K_3EDTA Plasma, and microsomes of human origin. There is no cross reactivity with human Calreticulin, Hsp90α, Hsp90β, Hsp70, canine Grp94, hamster Grp78, and bovine Hsc70.

Dilutional Linearity

To determine possible interference from the sample matrix, the indicated sample types were serially diluted into assay buffer. The concentrations of PDI 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.

CL: Cell Lysate **MS:** Microsomes

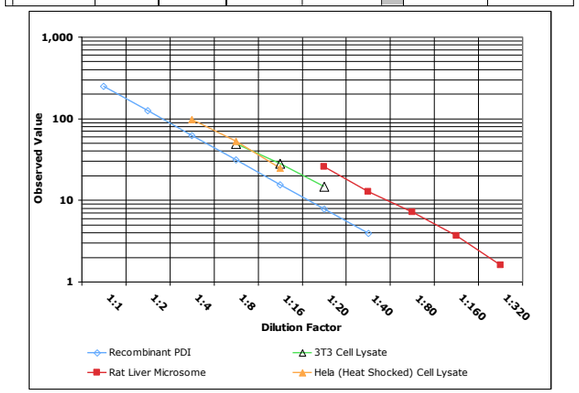
*Cell lysate (prepared in Extraction Reagent) was diluted at least 1:2 in Assay Buffer for levels to be within the dynamic range of the assay.

**Microsomes were diluted 1:10 in 1x Extraction Reagent and further diluted 1:4 in Assay Buffer for levels to be within the dynamic range of the assay.

Parallelism

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

Dilution Factor	Hela CL	C6 CL	3T3 CL	Plasma (Rat)	Dilution Factor	Liver MS (Rat)
1:2	79%*	62%*	109%*	109%**	1:20	61%***
1:4	99%	79%	121%	121%	1:40	101%
1:8	107%	89%	100%	100%	1:80	100%
1:16	100%	94%	---	---	1:160	113%
1:32	---	100%	---	---	1:320	116%
1:32	---				1:640	100%



Calculation of Results

Several options are available for the calculation of the relative levels of PDI 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	ADI-80-1930
PBS Concentrate	120 mL	ADI-80-1927
BSA Solution (10%)	50 mL	ADI-80-1928
Tween-20 Solution (10%)	30 mL	ADI-80-1929
Extraction Reagent (5x)	10 mL	ADI-80-1526
Wash Buffer Concentrate	100 mL	ADI-80-1287
SA-HRP	12.5 µg/vial	ADI-80-1896

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

Catalog No. 25-0637 Last Revised: 08/15/16