PRDX4 (Human) ELISA Kit

Catalog Number KA2121

96 assays

Version: 34

Intended for research use only

www.abnova.com

Table of Contents

Introduction

Intended Use

The PRDX4 (Human) ELISA Kit is to be used for the in vitro quantitative determination of human Prx4 in human serum, human plasma, cell lysate and buffered solution. The assay will recognize both native and recombinant human Prx4.
This kit has been configured for research use only and is not to be used in diagnostic procedures.

Background

Peroxiredoxin (Prx) is a growing peroxidase family, whose mammalian members have been known to connect with cell proliferation, differentiation, and apoptosis. Many isoforms (about 50 proteins), collected in accordance to the amino acid sequence homology, particularly amino-terminal region containing active site cysteine residue, and the thiol-specific antioxidant activity, distribute throughout all the kingdoms. Among them, mammalian Prx consists of 6 different members grouped into typical 2-Cys, atypical 2-Cys Prx, and 1-Cys Prx. Except Prx VI belonging to 1-Cys Prx subgroup, the other five 2-Cys Prx isotypes have the thioredoxin-dependent peroxidase (TPx) activity utilizing thioredoxin, thioredoxin reductase, and NADPH as a reducing system. Mammalian Prxs are 20 - 30 kilodalton in molecular size and vary in subcellular localization: Prx I, II, and VI in cytosol, Prx III in mitochondria, Prx IV in ER and secretion, Prx V showing complicated distribution including peroxisome, mitochondria and cytosol (1).

Principle of the Assay

The design of this assay is based on a sandwich Enzyme-Linked Immunosorbent Assay (ELISA). The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific to human Prx4. Samples are pippetted into these wells. Nonbound Prx4 and other components of the sample should be removed by washing, then biotin-conjugated monoclonal antibody specific to Prx4 added. In order to quantitatively determine the amount of Prx4 present in the sample, Avidin conjugated to Horseradish Peroxidase (HRP) should be added to each microplate well. The final step, a TMB-substrate solution added to each well. Finally, a sulfuric acid solution is added and the resulting yellow colored product is measured at 450 nm. Since the increases in absorbency is directly proportional to the amount of captured Prx4.

General Information

Materials Supplied

List of component

Component	Amount
96 Well Plate (in aluminium foil bag with desiccant) Human Peroxiredoxin IV microtiter plate: A plate using break-apart strips coated with a mouse monoclonal antibody specific to human Peroxiredoxin IV.	96 (8x12) wells
Incubation Buffer	30 mL
Washing Buffer (20x)	25 mL x 2
Standard Protein (lyophilized): Recombinant human Peroxiredoxin IV.	1 vial
Standard/Sample/Secondary Antibody Dilution Buffer	25 mL
Secondary Antibody (lyophilized): Biotinylated anti human Peroxiredoxin IV antibody.	1 vial
AV-HRP: Avidin linked Horseradish Peroxidase (HRP, enzyme).	150 µL
AV-HRP Dilution Buffer	25 mL
Substrate (Stabilized chromogen): Tetramethylbenzidine (TMB) solution.	15 mL
Stop Solution: 1 N solution of sulfuric acid (H_2SO_4).	15 mL
Plate sealers: Adhesive sheet.	2 slides

Note: Do not mix or interchange different reagents from various kit lots.

Storage Instruction

All kit components of this kit are stable at 2 to 8°C. Any unused reconstituted standard should be discarded or frozen at -70°C. Standard can be frozen and thawed one time only without loss of immunoreactivity.

Materials Required but Not Supplied

- ✓ Microtiter plate reader capable of measurement at or near 450 nm.
- ✓ Calibrated, adjustable precision pipettes, preferably with disposable plastic tips (A manifold multi-channel pipette is desirable for large assays.)
- ✓ Distilled or deionized water
- ✓ Data analysis and graphing software
- ✓ Vortex mixer
- ✓ Polypropylene tubes for diluting and aliquoting standard
- ✓ Absorbent paper towels
- ✓ Calibrated beakers and graduated cylinders of various sizes

Precautions for Use

- ✓ Stop solution: This reagent is an irritant to eyes, skin and mucous membranes. Avoid contact with eyes, skin and clothing. Wear suitable protective clothing, gloves and eye protection. In the event of contact with eyes or skin, wash immediately with plenty of water.
- ✓ Standard protein and 2nd Antibody containing Sodium Azide as a preservative.

Assay Protocol

Reagent Preparation

- Human Prx4 standard
  1. Reconstitute the lyophilized Human Prx4 standard by adding 1 mL of Standard/Sample/Secondary antibody Dilution Buffer to make the 20 ng/mL standard stock solution. Allow solution to sit at RT for 5 minutes, then gently vortex to mix completely. Use within one hour of reconstituting.
  2. Prepare 1 mL of 5000 pg/mL top standard by adding 250 µL of the above stock solution in 750 µL of Standard/Sample/Secondary antibody Dilution Buffer. Perform 2-fold serial dilutions of the top standards to make the standard curve within the range of this assay (78.13 pg/mL ~ 5000 pg/mL) as below. Standard/Sample/Secondary antibody Dilution Buffer serves as the zero standard (0 pg/mL).

Standard	Add	Into
5000 pg/mL	250.0 µL of the std. (20 ng/mL)	750.0 µL of the Standard/Sample/Secondary antibody Dilution Buffer
2500 pg/mL	500 µL of the std. (5000 pg/mL)	500.0 µL of the Standard/Sample/Secondary antibody Dilution Buffer
1250 pg/mL	500 µL of the std. (2500 pg/mL)	500.0 µL of the Standard/Sample/Secondary antibody Dilution Buffer
625 pg/mL	500 µL of the std. (1250 pg/mL)	500.0 µL of the Standard/Sample/Secondary antibody Dilution Buffer
312.5 pg/mL	500 µL of the std. (625 pg/mL)	500.0 µL of the Standard/Sample/Secondary antibody Dilution Buffer
156.25 pg/mL	500 µL of the std. (312.5 pg/mL)	500.0 µL of the Standard/Sample/Secondary antibody Dilution Buffer
78.13 pg/mL	500 µL of the std. (156.25 pg/mL)	500.0 µL of the Standard/Sample/Secondary antibody Dilution Buffer
0 pg/mL	1.0 mL of the Standard/Sample/Secondary antibody Dilution Buffer	

- Secondary Antibody

  100X secondary antibody solution can be made by adding 150 µL Standard/Sample/Secondary antibody dilution buffer in the vial.
  1. Equilibrate to room temperature, mix gently.
  2. Mix 20 µL Secondary Antibody concentrated solution (100X) + 1.98 mL Standard/Sample/Secondary Antibody dilution buffer. (Sufficient for two 8-well strip, prepare more if necessary) Label as “Working Secondary antibody Solution”.
  3. Return the unused Secondary Antibody concentrated solution to the refrigerator.

- AV-HRP
  1. Equilibrate to room temperature, mix gently.
  2. Mix 20 µL AV-HRP concentrated solution (100X) + 1.98 mL AV-HRP dilution buffer. (Sufficient for two 8-well strip, prepare more if needed). Label as “Working AV-HRP Solution”.
  3. Return the unused AV-HRP concentrated solution to the refrigerator.
- Washing buffer
  1. Equilibrate to room temperature, mix to re-dissolve any precipitated salt.
  2. Mix 0.5 volume Wash buffer concentrate solution (20X) + 9.5 volumes of deionized water. Label as “Working Washing Solution”.
  3. Store both the concentrated and the Working Washing Solution in the refrigerator.

  * Directions for washing
  1. Fill the wells with 300 µL of “Working Washing Buffer”. Let soak for 1 to 3 minutes and then all residual wash-liquid must be drained from the wells by aspiration (taking care not to scratch the inside of the well) or decantation, followed by forceful tapping of the plate on absorbent paper. Never insert absorbent paper directly into the wells.

     If using an automated washer, the operating instructions for washing equipment should be carefully followed.
  2. Incomplete washing will adversary affects the assay and renders false results.
  3. It is recommended to use laboratory tape to hold plate strips to the plate frame while performing the plate washing to avoid strips coming free of the frame.

Sample Preparation

Blood should be collected by veinpuncture. For plasma samples, blood may be drawn into tubes containing sodium citrate or heparin, EDTA. The serum or plasma should be separated from the coagulated or packed cells by centrifugation. Specimens may be shipped at room temperature and then stored refrigerated at 2-8℃ if testing is to take place within one week after collection. If testing is to take place later than one week, specimens should be stored at -20℃. Avoid repeated freeze/thawing.

Assay Procedure

- ✓ Allow all reagents to reach room temperature before use. Gently mix all liquid reagents prior to use.
- ✓ All standards, controls and samples should be run in duplicate for confirmation of reproducibility.
- ✓ A standard curve must be run with each assay.
- ✓ If particulate matter is present in the analyte, centrifuge or filter prior to analysis.
- ✓ Maintain a consistent order of components and reagents addition from well to well. This ensures equal incubation times for all wells.

1. Determine the number of 8-well strips needed for assay. Insert these in the flame(s) for current use (Re-bag extra strips and frame. Refrigerate for further use).

2. Add 300 µL of Incubation buffer to all wells and incubate the plate for 5 minutes at room temperature.
3. Thoroughly aspirate or decant the solution from the wells. Wash wells 1 times (See “Directions for washing”).
4. For the standard curve, add 100 µL of the standard to the appropriate microtiter wells. Add 100 µL of the Standard/Sample/Secondary antibody Dilution Buffer to zero wells.
5. Serum and plasma require at least 20 fold dilution in the Standard/Sample/Secondary antibody Dilution Buffer. And add 100 µL of samples to each wells.
6. Cover the plate with the plate cover and incubate for 2 hours at 37°C.
7. Thoroughly aspirate or decant the solution from the wells. Wash the wells 3 times (See “Directions for washing”).
8. Pipette 100 µL of “Working Secondary Antibody Solution” into each well.
9. Cover the plate with the plate cover and incubate for 1 hour at room temperature.
10. Thoroughly aspirate or decant the solution from the wells. Wash the wells 3 times (See “Directions for washing”).
11. Add 100 µL “Working AV-HRP Solution” to each well.
12. Cover the plate with the plate cover and incubate for 30 minutes at room temperature.
13. Thoroughly aspirate or decant the solution from the wells. Wash the wells 3 times (See “Directions for washing”).
14. Add 100 µL of Substrate to each well. The liquid in the wells should begin to turn blue.
15. Incubate the plate at room temperature.
    - ✓ Do not cover the plate with aluminum foil, or color may develop.
      The incubation time for chromogen substrate is often determined by the microtiter plate reader used. O.D. values should be monitored and the substrate reaction stopped before O.D. of the positive wells exceeds the limits of the instrument. O.D. values at 450 nm can only be read after the Stop Solution has been added to each well.
    - ✓ Because the Substrate is light sensitive, avoid the remained Substrate solution prolonged exposure to light.
    - ✓ Typically, reaction is stopped 5~10 minutes after treatment of Substrate, but this time can be adjusted as the user desires.
16. Add 100 µL of Stop Solution to each well. The solution in the wells should change from blue to yellow.
17. Read the absorbance of each well at 450 nm. Read the plate within 20 minutes of adding the Stop Solution.
18. Plot on graph paper the absorbance of the standard against the standard concentration (Optimally, the background absorbance can be subtracted from all data points, including standards, unknowns and controls, prior to plotting.). Draw a smooth curve through these points to construct the standard curve.
19. Read the human Prx4 concentrations for the unknown samples and controls from the standard curve plotted in step 18. Multiply value(s) obtained for the unknown sample by the dilution factor (Samples producing signals greater than that of the highest should be further diluted in the Standard/Sample/Secondary antibody Dilution Buffer).

Data Analysis

Calculation of Results

- Typical result

  The standard curve below is for illustration only and should not be used to calculate results in your assay.

  A standard curve must be run with each assay.

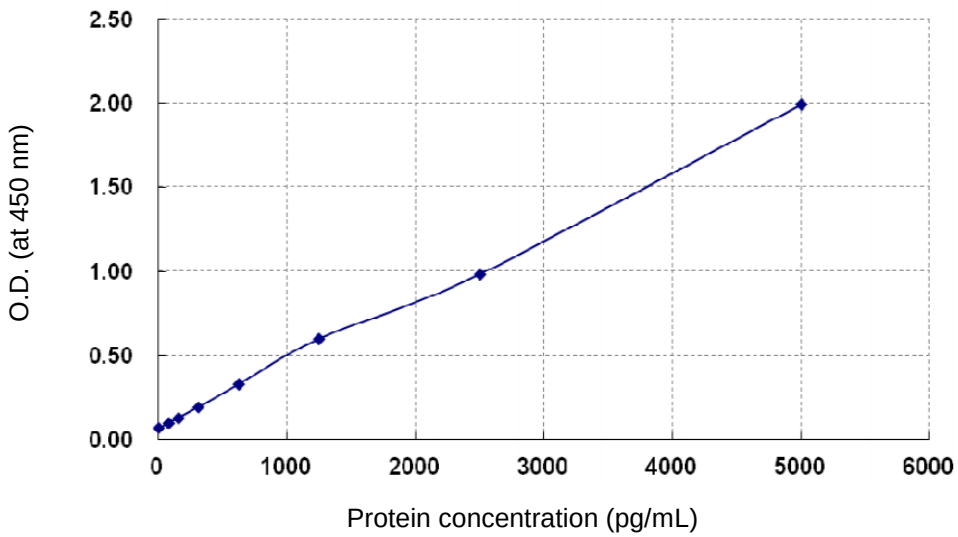
Standard human Prx4 (pg/mL)	Optical Density (at 450 nm)
0	0.065
78.13	0.099
156.25	0.129
312.5	0.194
625	0.329
1250	0.600
2500	0.985
5000	1.991

- Limitation

✓ Do not extrapolate the standard curve beyond the 5000 pg/mL standard point.

✓ Other buffers and matrices have not been investigated.

✓ The rate of degradation of native human Peroxiredoxin IV in various matrices has not been investigated.



TMB reaction incubate at room temperature for 5 min

Performance Characteristics

- Sensitivity

  The minimal detectable dose of human Prx4 was calculated to be 6.77 pg/mL, by subtracting two standard deviations from the mean of 12 zero standard replicates (ELISA buffer, S0) and intersecting this value with the standard curve obtained in the same calculation.

N	1	2	3	4	5	6	7	8	9	10	11	12
ZERO	0.059	0.057	0.056	0.059	0.060	0.060	0.057	0.057	0.058	0.057	0.059	0.059

Average	SD	LLD	LLD mean (pg/mL)
0.0582	0.0013	0.061	6.77

- Specificity

  The following substances were tested and found to have no cross-reactivity: human Prx1, human Prx2, human Prx3, human Prx5, human Prx6, mouse Prx4, human MPO, human SOD1, human SOD2, human SOD3

- Precision

  Within-Run (Intra-Assay)

Mean (pg/mL)	SD (ng/mL)	CV (%)
73.15	2.93	4.00
148.43	9.52	6.41
313.57	12.71	4.05
633.82	33.66	5.31
1269.21	63.43	5.00

(n=6)

Between-Run (Inter-Assay)

Mean (pg/mL)	SD (ng/mL)	CV (%)
72.58	4.41	6.08
145.10	8.16	5.62
295.45	11.89	4.03
597.16	30.02	5.03
1185.83	51.38	4.33

(n=8)

- Recovery

  Recovery on addition is 98.21~114.62% (mean 105.71%)

Analyte added (pg/mL)	Serum (pg/mL)	Recovery (%)	Plasma (pg/mL)	Recovery (%)
312.5	342.00	107.59	331.69	104.36
1250	1364.38	112.10	1395.16	114.62
2500	2646.69	101.32	2742.44	104.98
5000	5071.12	102.49	4859.11	98.21

Resources

Troubleshooting

Problem	Possible Cause	Solution
High signal and background in all wells	• Insufficient washing	• Increase number of washes • Increase time of soaking between in wash
	• Too much AV-HRP	• Check dilution, titration
	• Incubation time too long	• Reduce incubation time
	• Development time too long	• Decrease the incubation time before the stop solution is added
No signal	• Reagent added in incorrect order, or incorrectly prepared	• Review protocol
	• Standard has gone bad (If there is a signal in the sample wells)	• Check the condition of stored standard
	• Assay was conducted from an incorrect starting point	• Reagents allows to come to 20~30°C before performing assay
Too much signal – whole plate turned uniformly blue	• Insufficient washing – unbound AV-HRP remaining	• Increase number of washes carefully
	• Too much AV-HRP	• Check dilution
	• Plate sealer or reservoir reused, resulting in presence of residual AV-HRP	• Use fresh plate sealer and reagent reservoir for each step
Standard curve achieved but poor discrimination between point	• Plate not developed long enough	• Increase substrate solution incubation time
	• Improper calculation of standard curve dilution	• Check dilution, make new standard curve
No signal when a signal is expected, but standard curve looks fine	• Sample matrix is masking detection	• More diluted sample recommended
Samples are reading too high, but standard curve is fine	• Samples contain protein levels above assay range	• Dilute samples and run again
Edge effect	• Uneven temperature around work surface	• Avoid incubating plate in areas where environmental conditions vary • Use plate sealer

References

1. Wood, Z. A. et al. (2003) Trends Biochem Sci. 28(1):32-40
2. Rhee Sue Goo et al. (2001) IUBMB Life. 52:35-41

Plate Layout

	A	B	C	D	E	F	G	H
12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								