PRDX3 (Human) ELISA Kit

Catalog Number KA0531

96 assays

Version: 04

Intended for research use only

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Introduction

Intended Use

The PRDX3 (Human) ELISA Kit is to be used for the in vitro quantitative determination of human Prx3 in human mitochondrial fraction, human serum, cell lysate or buffered solution. The assay will recognize both native and recombinant human Prx3.
This kit has been configured for research use only and is not to be used in diagnostic procedures.

Background

Organisms living under aerobic conditions have developed various anti-oxidative mechanisms to protect them from damage by reactive oxygen species (ROS). Peroxiredoxin (Prx) is a growing anti-oxidative protein family that has been identified six members in mammals. They share a common reactive Cys residue in the N-terminal region, and are capable of serving as a peroxidase and involve thioredoxin and/or glutathione as the electron donor. Prx1 to Prx4 have an additional Cys residue in the conserved C-terminal region, and are cross members as judged by the amino acid sequence similarity. Prx5 also contains an additional Cys in its C-terminal region that is less conserved. On the other hand, Prx6 has only one unique Cys. These Prx family members are distributed in subcellular localization, Prx1, 2, and 6 in cytosol, Prx3 in mitochondria, Prx4 in ER and secretion, Prx5 showing complicated distribution including peroxisome, mitochondria and cytosol, all of which are potential sites of ROS production. In addition to their role as a peroxidase, however, a body of evidence has accumulated to suggest that individual members also serve divergent functions, which are associated with various biological processes such as the detoxification of oxidants, cell proliferation, differentiation and gene expression. It would be expected that these functions might not necessarily depend on peroxidase activity and, therefore, it seems likely that the divergence is due to unique molecular characteristics intrinsic to each member.

The specific localization of Prx3 in mitochondria together with the identification of its mitochondria-specific electron suppliers, namely thioredoxin 2 and thioredoxin reductase 2, suggest that these three proteins might provide a primary line of defense against H_2O_2 produced by the mitochondrial respiratory chain. Furthermore, Prx3 expression is induced by oxidants in the cardiovascular system and is thought to play a role in the antioxidant defense system and homeostasis within mitochondria.

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 Prx3. Samples are pipetted into these wells. Nonbound Prx3 and other components of the sample should be removed by washing, then biotin-conjugated monoclonal antibody specific to Prx3 added. In order to quantitatively determine the amount of Prx3 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. And 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 Prx3.

General Information

Materials Supplied

List of component

Component	Amount
Human Prx3 microtiter plate (in aluminium foil bag with desiccant): A plate using break-apart strips coated with a mouse monoclonal antibody specific to human Prx3.	96 (8x12) wells
Incubation Buffer	30 mL
Washing Buffer (20x)	25 mL x 2
Standard Protein (lyophilized): Recombinant human Prx3.	1 Glass vial
Standard/Sample Dilution Buffer	25 mL
Secondary Antibody (lyophilized): Biotinylated anti human Prx3 antibody.	1 Glass vial
AV-HRP (100x): Avidin linked Horseradish Peroxidase (HRP, enzyme).	150 µL
Secondary Antibody/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 sealer: Adhesive sheet.	2 slices

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 Prx3 standard

1. Reconstitute the lyophilized human Prx3 standard by adding 1 mL of Standard/Sample Dilution Buffer to make the 1 µg/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 32 ng/mL top standard by adding 32 µL of the above stock solution in 968 µL of Standard/Sample Dilution Buffer. Perform 2-fold serial dilutions of the top standards to make the standard curve within the range of this assay (2 ng/mL ~ 32 ng/mL) as below. Standard/Sample Dilution Buffer serves as the zero standard (0 ng/mL).

Standard	Add	Into
32 ng/mL	32.0 µL of the std. (1 µg/mL)	968.0 µL of the Standard/Sample Dilution Buffer
16 ng/mL	500 µL of the std. (32 ng/mL)	500.0 µL of the Standard/Sample Dilution Buffer
8 ng/mL	500 µL of the std. (16 ng/mL)	500.0 µL of the Standard/Sample Dilution Buffer
4 ng/mL	500 µL of the std. (8 ng/mL)	500.0 µL of the Standard/Sample Dilution Buffer
2 ng/mL	500 µL of the std. (4 ng/mL)	500.0 µL of the Standard/Sample Dilution Buffer
0 ng/mL	1.0 mL of the Standard/Sample Dilution Buffer	

✓ Secondary Antibody

100X secondary antibody solution can be made by adding 150 µL secondary antibody/AV-HRP dilution buffer in the vial.

1. Equilibrate to room temperature, mix gently.
2. Mix 20 µL Secondary Antibody concentrated solution (100X) + 1.98 mL Secondary Antibody/AV-HRP 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 Secondary Antibody/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 venipuncture. 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°C if testing is to take place within one week after collection. If testing is to take place later than on week, specimens should be stored at -20°C. 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 frame(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 2 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 Dilution Buffer to zero wells.
5. Serum require at least 30 fold dilution in the Standard/Sample Dilution Buffer. And add 100 µL of samples

to each well.

6. Cover the plate with the plate cover and incubate for 2 hours at room temperature.
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 Prx3 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 standard should be further diluted in the Standard/Sample 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 Prx3 (ng/mL)	Optical Density (at 450 nm)
0	0.059
2.00	0.112
4.00	0.210
8.00	0.480
16.00	1.206
32.00	2.514

Note: There is irregular characteristic in Prx3, oligomerization. So at full range, the linearity is not typical as other proteins. In other word, the phenomenon is shown that the standard curve is bent in the middle range. So to quantify your protein more precisely, we recommend that user choose the one section between two-divided ranges, which include O.D. of your protein and that calculate concentration in the chosen linearity section.

✓ Limitation

- Do not extrapolate the standard curve beyond the 32 ng/mL standard point.
- Other buffers and matrices have not been investigated.
- The rate of degradation of native human Prx3 in various matrices has not been investigated.

Performance Characteristics

✓ Sensitivity

The minimal detectable dose of human Prx3 was calculated to be 2 ng/mL, by subtracting two standard deviations from the mean of 10 zero standard replicates (ELISA buffer, S0) and intersecting this value with the standard curve obtained in the same calculation.

✓ Specificity

The following substances were tested and found to have no cross-reactivity: human Prx1, Prx2, prx4, Prx5, Prx6, mouse Prx3 and rat Prx3.

✓ Precision

- Within-Run (Intra-Assay)

(n=5)

Mean (ng/mL)	SD (ng/mL)	CV (%)
3.39	0.26	7.6
6.78	0.51	7.5
15.89	0.39	2.5
32.30	0.78	2.4

- Between-Run (Inter-Assay)

(n=6)

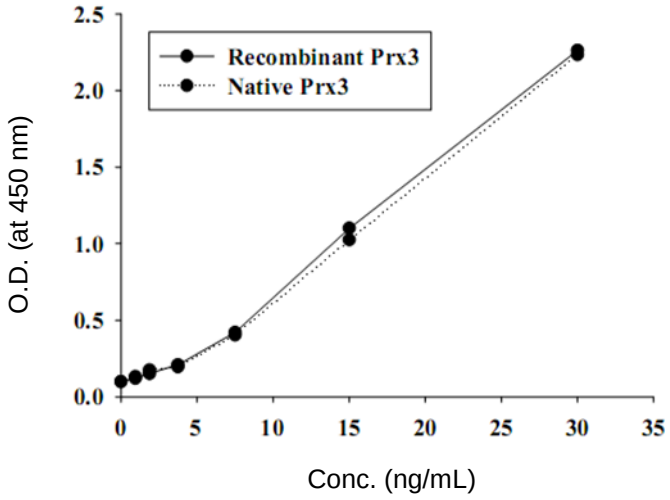
Mean (ng/mL)	SD (ng/mL)	CV (%)
3.64	0.15	4.0
7.06	0.16	2.3
16.06	0.49	3.1
32.18	0.75	2.3

✓ Recovery

Recovery on addition is 92.9~103.6% (mean 98.2%)

Recovery on dilution is 85.0~108.1% (mean 98.1%)

✓ Parallelism



Native human Prx3 from HeLa cell lysate was serially diluted in Standard/Sample dilution buffer. The optical density of each dilution was plotted against the recombinant human Prx3 standard curve.

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. Sue Goo Rhee et al. Peroxiredoxins: a historical overview and speculative preview of novel mechanisms and emerging concepts in cell signaling. (2005) Free Radic Biol Med. 38(12):1543-52.
2. Fujii J, Ikeda Y. Advances in our understanding of peroxiredoxin, a multifunctional, mammalian redox protein. (2002) Redox Rep. 7(3):123-30.
3. Sue Goo Rhee et al. Peroxiredoxin, a novel family of peroxidases. (2001) IUBMB Life 52(1-2):35-41.
4. Tong-Shin Chang et al. Peroxiredoxin III, a mitochondrion-specific peroxidase, regulates apoptotic signaling by mitochondria. (2004) J Biol Chem. 279(40):41975-84.
5. Larisa Nonn et al. Increased expression of mitochondrial peroxiredoxin-3 (thioredoxin peroxidase-2) protects cancer cells against hypoxia and drug-induced hydrogen peroxide-dependent apoptosis. (2003) Mol Cancer Res. 1(9):682-9.
6. Zachary A. Wood et al. Structure, mechanism and regulation of peroxiredoxins. (2003) TIBS 28(1): 32-40.

Plate Layout

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