ab185983 – Peroxiredoxin 1 (PRX1) Human ELISA Kit

Instructions for Use

For the quantitative measurement of Peroxiredoxin 1 (PRX1) in Human cell and tissue extracts.

This product is for research use only and is not intended for diagnostic use.

Version 1 Last Updated 31 August 2020

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1. BACKGROUND

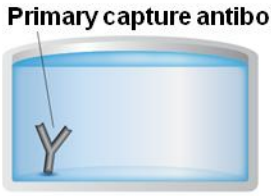
Peroxiredoxin 1 (PRX1) Human in vitro ELISA kit is designed for the accurate quantitative measurement of Peroxiredoxin 1 protein in Human cell and tissue extracts.

This assay employs an antibody specific for anti-PRX1 coated on the well a 96- well microplate. Standards and samples are pipetted into the wells and any PRX1 present in the sample is bound by the immobilized antibody. The wells are washed to remove any unbound sample and a PRX1 detector antibody is added. After washing an HRP label is pipetted to the wells and incubated. After incubation, the wells are washed to remove unbound material. HRP substrate is added and during incubation is catalyzed by HRP, generating blue coloration. This reaction is then stopped by addition of Stop Solution completing any color change from blue to yellow. Signal is generated proportionally to the amount of bound analyte and the intensity is measured at 450 nm. Optionally, instead of the endpoint reading, development of HRP can be recorded kinetically at 600 nm.

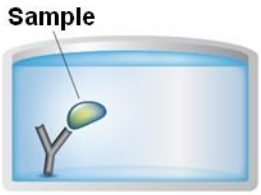
Peroxiredoxins are a widely-expressed family of antioxidant enzymes involved in redox regulation and oxidative stress defense. Their ability to reduce hydrogen peroxide by utilizing thioredoxin as an electron donor is used by cells to regulate intracellular hydrogen peroxide levels. Additionally, the inactivation of peroxiredoxins by high levels of hydrogen peroxide and the ATP-dependent reactivation by sulfiredoxin indicates peroxiredoxins have a role in redox signaling. Peroxiredoxin isoforms are grouped into two categories, 1-Cys and 2-Cys, based on the number of redox-active cysteine residues used in their catalytic mechanisms. The peroxiredoxin isoforms can also be characterized by their subcellular locations where PRX1, 2 and 6 are found in cytosol, PRX3 within mitochondria, and PRX4 is secreted and found in the ER.

Specifically, PRX1's regeneration by reduction with thioredoxin but not glutathione signifies the isoform's unique function as a regulator of hydrogen peroxide levels within cells.

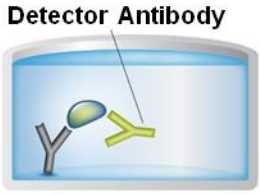
2. ASSAY SUMMARY



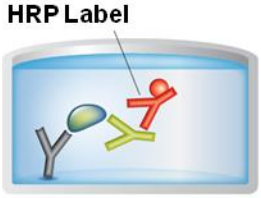
Remove appropriate number of antibody coated well strips. Equilibrate all reagents to room temperature. Prepare all the reagents, samples, and standards as instructed.



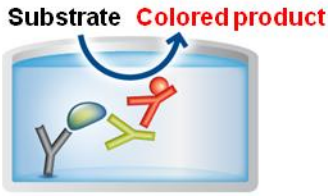
Add standard or sample to each well used. Incubate at room temperature.



Aspirate and wash each well. Add prepared Detector Antibody to each well. Incubate at room temperature.



Aspirate and wash each well. Add prepared HRP label. Incubate at room temperature.



Aspirate and wash each well. Add HRP Substrate to each well and incubate. Add Stop Solution at a defined endpoint. Alternatively, record color development kinetically after HRP substrate addition

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at +2-8ºC immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in sections 9 & 10.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
10X Wash Buffer	40 mL	+2-8ºC
5X Cell Extraction Buffer PTR	10 mL	+2-8ºC
Detergent	1 mL	+2-8ºC
10X Blocking Buffer	6 mL	+2-8ºC
PRX1 Microplate (12 x 8 well strips)	96 wells	+2-8ºC
PRX1 Human Lyophilized Recombinant Protein	1 Vial	+2-8ºC
10X PRX1 Detector Antibody	1 mL	+2-8ºC
10X HRP Label	1 mL	+2-8ºC
HRP Development Solution	12 mL	+2-8ºC
Stop Solution	12 mL	+2-8ºC

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 600 or 450 nm.
- Method for determining protein concentration (BCA assay recommended).
- Deionized water.
- Multi and single channel pipettes.
- PBS (1.4 mM KH2PO4, 8 mM Na2HPO4, 140 mM NaCl, 2.7 mM KCl, pH 7.3).
- Tubes for standard dilution.
- Plate shaker for all incubation steps (optional).
- Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors) and/or phosphatase inhibitors.

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not use kit or components if it has exceeded the expiration date on the kit labels.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

8. TECHNICAL HINTS

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.
- As a guide, typical ranges of sample concentration for commonly used sample types are shown below in Sample Preparation (section 11).
- All samples should be mixed thoroughly and gently.
- Avoid multiple freeze/thaw of samples.
- Incubate ELISA plates on a plate shaker during all incubation steps (optional).
- When generating positive control samples, it is advisable to change pipette tips after each step.
- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.

9. REAGENT PREPARATION

Equilibrate all reagents to room temperature (18-25°C) prior to use.

9.1 1X Wash Buffer

Prepare 1X Wash Buffer by adding 40 mL 10X Wash Buffer to 360 mL deionized water. Mix gently and thoroughly.

9.2 Incubation Buffer

Prepare Incubation Buffer by adding 6 mL 10X Blocking Buffer to 54 mL 1X Wash Buffer. Mix gently and thoroughly.

9.3 1X PRX1 Detector Antibody

Prepare 1X PRX1 Detector Antibody by diluting the 10X PRX1 Detector Antibody 10-fold with Incubation Buffer immediately prior to use. Prepare 500 µL 1X PRX1 Detector Antibody for each 8 well strip used

9.4 1X HRP Label

Prepare 1X HRP Label by diluting the 10X HRP Label 10-fold with 1X Incubation Buffer immediately prior to use. Prepare 500 µL 1X HRP Label for each 8 well strip used.

9.5 1X Cell Extraction Buffer PTR + Detergent

Prepare 1X Cell Extraction Buffer PTR + Detergent by adding 4 mL 5X Cell Extraction Buffer PTR and 100 µL Detergent to 15.9 mL deionized water.

Alternative – If sample becomes viscous or difficult to pipette during extraction, the Detergent may be added to the 1X Cell Extraction Buffer PTR after extraction of cells or tissue. Extraction efficiency may be lower.

- After opening, the unused Incubation Buffer and 1X Extraction Buffer PTR + Detergent should be stored at -20°C.
- If the Detergent has precipitates or is solid, slowly warm until completely dissolved.

10. STANDARD PREPARATION

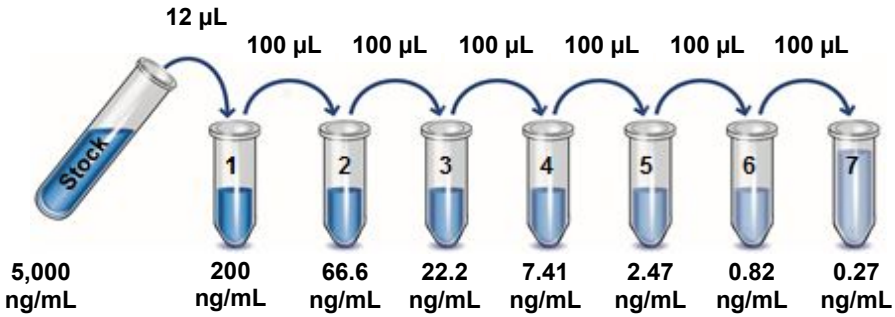
Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of positive controls for every use.

10.1 Reconstitute the standard sample by adding 100 µL deionized water to PRX1 Human Lyophilized Recombinant Protein by pipette. Allow to sit for 1 minute and gently mix. This 5,000 ng/mL **Stock Standard** Solution. Any remaining Stock Standard should be aliquoted and stored at -80°C if not immediately used.

10.2 Label eight tube, Standards 1– 8.

10.3 Add 288 µL 1X Cell Extraction Buffer PTR + Detergent into tube number 1 and 200 µL 1X Cell Extraction Buffer PTR + Detergent into numbers 2-8.

10.4 Use the Stock Standard to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:



11. SAMPLE PREPARATION

TYPICAL SAMPLE DYNAMIC RANGE -

Typical working ranges	
Sample Type	**Range**
HeLa Cell Extract	0.1 – 10 µg/mL
Human Heart Homogenate	Not detected

11.1 Preparation of extracts from cell pellets

11.1.1 Collect non adherent cells by centrifugation or scrape to collect adherent cells from the culture flask. Typical centrifugation conditions for cells are 500 x g for 5 minutes at 4ºC.

11.1.2 Rinse cells twice with PBS.

11.1.3 Solubilize cell pellet at $2x10^7$/mL in 1X Cell Extraction Buffer PTR + Detergent.

11.1.4 Incubate on ice for 20 minutes. Centrifuge at 18,000 x g for 20 minutes at 4°C. Transfer the supernatants into clean tubes and discard the pellets.

11.1.5 Add 1 part Detergent for 9 parts of extracted lysate. For example, if 900 µL of supernatant extract is recovered from the previous step, add 100µL of Detergent. It is important to add the Detergent before diluting the samples further.

11.1.6 Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

11.2 **Preparation of extracts from adherent cells by direct lysis (alternative protocol)**

11.2.1 Remove growth media and rinse adherent cells 2 times in PBS.

11.2.2 Solubilize the cells by addition of 1X Cell Extraction Buffer PTR directly to the plate (use 0.75 µL - 1.5 mL) 1X Cell Extraction Buffer per confluent 15 cm diameter plate).

11.2.3 Scrape the cells into a test tube and incubate the lysate on ice for 15 minutes. Centrifuge at 18,000 x g for 20 minutes at 4°C. Transfer the supernatants into clean tubes and discard the pellets.

11.2.4 Add 1 part Detergent for 9 parts of extracted lysate. For example, if 900 µL of supernatant extract is recovered from the previous step, add 100 µL of Detergent. It is important to add the E Detergent before diluting the samples further.

11.2.5 Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

11.3 **Preparation of extracts from tissue homogenates**

11.3.1 Tissue lysates are typically prepared by homogenization of tissue that is first minced and thoroughly rinsed in PBS to remove blood (dounce homogenizer recommended).

11.3.2 Homogenize 100 to 200 mg of wet tissue in 500 µL – 1 mL of 1X Cell Extraction Buffer PTR. For lower amounts of tissue adjust volumes accordingly.

11.3.3 Incubate on ice for 20 minutes. Centrifuge at 18,000 x g for 20 minutes at 4°C. Transfer the supernatants into clean tubes and discard the pellets.

11.3.4 Add 1 part Detergent for 9 parts of extracted lysate. For example, if 900 µL of supernatant extract is recovered from the previous step, add 100 µL of Detergent. It is important to add the Enhancer Solution before diluting the samples further.

11.3.5 Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

12. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused well strips should be returned to the plate packet and stored at 4°C.
- For each assay performed, a minimum of 2 wells must be used as the zero control.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Well effects have not been observed with this assay.

13. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

13.1 Prepare all reagents, working standards, and samples as directed in the previous sections.

13.2 The samples should be diluted within the working range of the assay in 1X Cell Extraction Buffer PTR + Detergent. As a guide, typical ranges of sample concentration are shown above (section 11). Add 50 µL of all samples or standards to appropriate wells. Include a 1X Cell Extraction Buffer PTR + Detergent as well as a background control.

13.3 Cover/seal the plate and incubate for two hours at room temperature. Shaking at 400 rpm is optional.

13.4 Wash each well with 3 x 300 µL 1X Wash Buffer. Wash by aspirating or decanting from wells then dispensing 300 µL 1X Wash Buffer into each well. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and blot it against clean paper towels to remove excess liquid.

13.5 Add 50 µL of 1X PRX1 Detector antibody (step 9.3) to each well used. Cover/seal the plate and incubate for one hour at room temperature. Shaking at 400 rpm is optional.

13.6 Wash each well three times with 1X Wash Buffer.

13.7 Add 50 µL of 1X HRP Label (step 9.4) to each well used. Cover/seal the plate and incubate shaking at 400rpm for one hour at room temperature.

13.8 Wash each well with 3 x 300 µL 1X Wash Buffer. Wash by aspirating or decanting from wells then dispensing 300 µL 1X Wash Buffer into each well. Complete removal of liquid at each step is essential for good performance. After the

last wash invert the plate and blot it against clean paper towels to remove excess liquid.

13.9 Add 50 µL HRP Development Solution to each well used and incubate for 3 minutes on a plate shaker set to 400 rpm.

13.10 Add 50 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm.

Alternative to 13.10 – 13.11: Instead of the endpoint reading at 450 nm, record the development of HRP Substrate kinetically. Immediately after addition of HRP Development Solution begin recording the blue color development with elapsed time in the microplate reader prepared with the following settings:

Mode:	Kinetic
Wavelength:	600 nm
Time:	up to 3 min
Interval:	10 sec - 1 min
Shaking:	Shake between readings

Note that an endpoint reading can also be recorded at the completion of the kinetic read by adding 100 µL Stop Solution to each well and recording the OD at 450 nm.

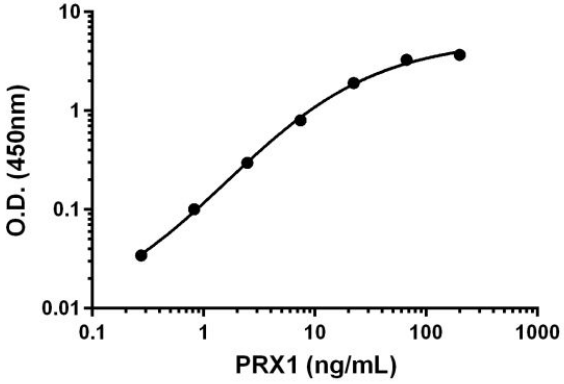
13.11 Analyze the data as described below.

14. CALCULATIONS

Subtract average zero standard reading from all readings. Average the duplicate readings of the positive control dilutions and plot against their concentrations. Draw the best smooth curve through these points to construct a standard curve. Most plate reader software or graphing software can plot these values and curve fit. A four parameter algorithm (4PL) usually provides the best fit, though other equations can be examined to see which provides the most accurate (e.g. linear, semi-log, log/log, 4 parameter logistic). Read relative protein concentrations for unknown samples from the standard curve plotted. Samples producing signals greater than that of the highest standard should be further diluted and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.

15. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.



Standard Curve Measurements				
Conc (ng/mL)	O.D.			Mean O.D.
	1	2	3	
0	0.151	0.132	0.130	0.138
0.27	0.182	0.166	0.169	0.172
0.82	0.244	0.242	0.231	0.239
2.47	0.439	0.431	0.435	0.435
7.41	0.964	0.926	0.934	0.941
22.22	2.039	2.024	2.089	2.051
66.67	3.423	3.396	3.449	3.423
200	3.811	3.841	3.833	3.828

Figure 1. Example of PRX1 protein standard curve. The PRX1 standard curve was prepared as described in Section 10. . Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

TYPICAL SAMPLE VALUES

SENSITIVITY -

The calculated minimal detectable dose (MDD) is 200 pg/mL**.** The MDD was determined by calculating the mean of zero standard replicates and adding 2 standard deviations then extrapolating the corresponding concentrations.

RECOVERY –

Three concentrations of Peroxiredoxin 1 Human recombinant protein were spiked in duplicate to the indicated biological matrix to evaluate signal recovery in the working range of the assay.

Sample Type	Average % Recovery	Range (%)
50% OF-HGDMEM	88	74 - 100
10% Normal Human Serum	82	70 - 97

LINEARITY OF DILUTION –

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Dilution	Interpolated PRX1 (ng/mL)	mO.D./min (600 nm)	% Expected Value
No Dilution	4.02	14.526	100
1:0.5	7.14	22.836	88.8
1:2	2.19	9.164	108.9
1:4	1.19	6.066	118.7

PRECISION –

	Intra-Assay	Inter-Assay
n=	3	3
%CV	4.15	7.3

16. ASSAY SPECIFICITY

This kit recognizes Human Peroxiredoxin 6 protein in Human cell and tissue extracts.

CROSS REACTIVITY -

The proteins listed below were prepared at 200 ng/mL and assayed for cross-reactivity. No significant cross-reactivity was observed.

Recombinant Human:

Peroxiredoxin 2

Peroxiredoxin 3

Peroxiredoxin 4

Peroxiredoxin 6

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17. TROUBLESHOOTING

Problem	Cause	Solution
Difficulty pipetting lysate; viscous lysate.	Genomic DNA solubilized	Prepare 1X Cell Extraction Buffer PTR (without detergent). Add enhancer to lysate after extraction.
Detergent is solid or shows precipitates.	Storage at low temperature caused component to precipitate.	Slowly warm component until it is fully dissolved again.
Poor standard curve	Inaccurate Pipetting	Check pipettes
	Improper standard dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions
	Contaminated wash buffer	Make fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store your reconstituted standards at -80°C, all other assay components 4°C. Keep substrate solution protected from light

18. **NOTES**

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)