PRDX4 (Human) ELISA Kit

Catalog Number KA6325

96 assays

Version: 01

Intended for research use only

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Table of Contents

Introduction

Intended Use

For the quantitative measurement of PRDX4 in Human biological samples.

Background

Regulates the activation of NF-kappa-B in the cytosol by a modulation of I-kappa-B-alpha phosphorylation.

Principle of the Assay

PRDX4 ELISA Kit (Human) is based on standard sandwich enzyme-linked immuno-sorbent assay technology. An antibody specific for PRDX4 has been pre-coated onto a 96-well plate (12 x 8 Well Strips). Standards or test samples are added to the wells, incubated and removed. A biotinylated detector antibody specific for PRDX4 is added, incubated and followed by washing. Avidin-Peroxidase Conjugate is then added, incubated and unbound conjugate is washed away. An enzymatic reaction is produced through the addition of TMB substrate which is catalyzed by HRP generating a blue color product that changes to yellow after adding acidic stop solution. The density of yellow coloration read by absorbance at 450 nm is quantitatively proportional to the amount of sample PRDX4 captured in the well.
UniProt: Q13162
Cross-Reactivity: No detectable cross-reactivity with other relevant proteins

General Information

Materials Supplied

List of component

Component	Amount	Storage conditions
PRDX4 Microplate	96 wells (12x8)	Store at -20°C for 6 months
PRDX4 Lyophilized Standard	2 vials	
100X Biotinylated PRDX4 Detector Antibody	120 µL	
100X Avidin-HRP Conjugate	120 µL	
Sample Diluent	20 mL	
Detector Antibody Diluent	12 mL	
Conjugate Diluent	12 mL	
25X Wash Buffer	30 mL	Store at 4°C for 6 months
Stop Solution	10 mL	
TMB Substrate	10 mL	

Storage Instruction

✓ Store components at -20°C for 6 months or until expiration date. Avoid any freeze/thaw cycles.

Materials Required but Not Supplied

✓ Microplate reader capable of reading absorbance at 450 nm.
✓ Automated plate washer (optional).
✓ Pipettes capable of precisely dispensing 0.5 µL through 1 mL volumes of aqueous solutions.
✓ Pipettes or volumetric glassware capable of precisely measuring 1 mL through 100 mL of aqueous solutions.
✓ New, clean tubes and/or micro-centrifuge tubes for the preparation of standards or samples.
✓ Absorbent paper or paper toweling.
✓ Distilled or deionized ultrapure water.
✓ 37°C Incubator (optional)

Precautions for Use

For research use only. Not for use in diagnostic procedures.

- Precautions

✓ Read instructions fully prior to beginning use of the assay kit.

✓ Any deviations or modifications from the described method or use of other reagents could result in a reduction of performance.

✓ Reduce exposure to potentially harmful substances by wearing personal protective lab equipment including lab coats, gloves and glasses.

- Technical application tips

✓ Do not mix or substitute components from other kits.

✓ To ensure the validity of experimental operation, it is recommended that pilot experiments using standards and a small selection of sample dilutions to ensure optimal dilution range for quantitation.

✓ Samples exhibiting OD measurements higher than the highest standard should be diluted further in the appropriate sample dilution buffers.

✓ Prior to using the kit, briefly spin component tubes to collect all reagents at the bottom.

✓ Replicate wells are recommended for standards and samples.

✓ Cover microplate while incubating to prevent evaporation.

✓ Do not allow the microplate wells dry at any point during the assay procedure.

✓ Do not reuse tips or tube to prevent cross contamination.

✓ Avoid causing bubbles or foaming when pipetting, mixing or reconstituting.

✓ Completely remove of all liquids when washing to prevent cross contamination.

✓ Prepare reagents immediately prior to use and do not store, with the exception of the top standard.

✓ Equilibrate all materials to ambient room temperature prior to use (standards exception).

✓ For optimal results for inter- and intra-assay consistency, equilibrate all materials to room temperature prior to performing assay (standards exception) and perform all incubations at 37°C.

✓ Pipetting less than 1 µL is not recommended for optimal assay accuracy.

✓ Once the procedure has been started, all steps should be completed without interruption. Ensure that all reagents, materials and devices are ready at the appropriate time.

✓ Incubation times will affect results. All wells should be handled in the same sequential order and time intervals for optimal results.

✓ Samples containing bilirubin, precipitates or fibrin strands or are hemolytic or lipemic might cause inaccurate results due to interfering factors.

✓ TMB Substrate is easily contaminated and should be colorless or light blue until added to plate. Handle carefully and protect from light.

Assay Protocol

Reagent Preparation

Equilibrate all materials to room temperature prior to use and use immediately.

Standard

- ✓ Prepare a fresh standard curve for each assay performed. Reconstituted standards cannot be stored for later use. For further directions, please refer to the Certificate of Analysis.

1X Biotinylated PRDX4 Detector Antibody

- ✓ Prepare the 1X Biotinylated PRDX4 Detector Antibody immediately prior to use by diluting the 100X Biotinylated PRDX4 Detector Antibody 1:100 with Detector Antibody Diluent.
- ✓ For each well strip to be used in the experiment (8-wells) prepare 1,000 µL by adding 10 µL of 100X Biotinylated PRDX4 Detector Antibody to 990 µL Detector Antibody Diluent.
- ✓ Mix thoroughly and gently. Hold no longer than 2 hours prior to using in procedure. Do not store at 1X concentration for future use.

1X Avidin-HRP Conjugate

- ✓ Prepare the 1X Avidin-HRP Conjugate immediately prior to use by diluting the 100X Avidin-HRP Conjugate 1:100 with Conjugate Diluent.
- ✓ For each well strip to be used in the experiment (8-wells) prepare 1,000 µL by adding 10 µL of 100X Avidin-HRP Conjugate to 990 µL Conjugate Diluent.
- ✓ Mix thoroughly and gently. Hold no longer than 2 hours prior to using in procedure. Do not store at 1X concentration for future use.

1X Wash Buffer

- ✓ If crystals have formed in the 25X Wash Buffer concentrate, equilibrate to room temperature and mix gently until crystals have completely dissolved.
- ✓ Add the entire 30 mL contents of the 25X Wash Buffer bottle to 720 mL of ultra-pure water to a clean > 1,000 mL bottle or other vessel.
- ✓ Seal and mix gently by inversion. Avoid foaming or bubbles.
- ✓ Store the 1X Wash Buffer at room temperature until ready to use in the procedure. Store the prepared 1X Wash Buffer at 4°C for no longer than 1 week. Do not freeze.

Microplate Preparation

- ✓ Micro-plates are provided ready to use and do not require rinsing or blocking.
- ✓ Unused well strips should be returned to the original packaging, sealed and stored at 4°C.
- ✓ Equilibrate microplates to ambient temperatures prior to opening to reduce potential condensation.

Sample Preparation

- ✓ Samples must be tested to determine if the kit is valid.
- ✓ Store samples to be assayed at 4°C for 24 hours prior being assayed.
- ✓ For long term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

General Sample Preparation Guidelines:

- ✓ Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature or overnight at 4°C before centrifugation for 15 minutes at 1,000 x g. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.
- ✓ Plasma - Collect plasma using EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1,000 x g at 4°C within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.
- ✓ Tissue Homogenates – Rinse 100 mg of tissue with 1X PBS, then homogenize in 1 mL of 1X PBS and store overnight at -20°C. Perform two freeze-thaw cycles to break the cell membranes, then centrifuge the homogenate for 5 minutes at 5,000 x g, 2-8°C. Remove the supernatant and assay immediately. Alternatively, aliquot and store samples at -20°C or -80°C. Centrifuge the sample again after thawing before the assay. Avoid repeated freeze-thaw cycles.
- ✓ Cell Lysates - Adherent cells should be detached with trypsin and then collected by centrifugation (suspension cells can be collected by centrifugation directly). Wash cells three times in cold PBS. Resuspend cells in PBS (1×) and ultrasonicate the cells 4 times (or freeze cells at ≤ -20°C. Thaw cells with gentle mixing. Repeat the freeze/thaw cycle 3 times.) Centrifuge at 1,500 x g for 10 minutes at 2 - 8°C to remove cellular debris.
- ✓ Cell culture supernatants and other biological fluids – Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

Recombinant Proteins: Due to the possibility of mismatching between antigen from other origin and antibody used in our kits (e.g. antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by our products

Sample dilution:

Target protein concentration must be estimated and appropriate sample dilution selected such that the final target protein concentration falls near the middle of the assay linear dynamic range. Samples exhibiting saturation should be further diluted.

- ✓ Prior to performing the full experiment, test a serially diluted representative sample.
  - a small pool of several samples can also be used with this same method if sample volume is limited.
- ✓ Dilute samples using Sample Diluent.
- ✓ Mix diluted samples gently and thoroughly.
- ✓ Pipetting less than 2 µL is not recommended for optimal assay accuracy.

Assay Procedure

Equilibrate all reagents and materials to ambient room temperature prior to use in the procedure. Place the desired number of coated strips into the holder.

Optimal results for intra- and inter-assay reproducibility will be obtained when performing incubation steps at 37°C as indicated below.

1. Determine the required number of wells and return any remaining unused wells and desiccant to the pouch.
2. Add 100 µL of serially titrated standards, diluted samples or blank into wells of the PRDX4 Microplate. At least two replicates of each standard, sample or blank is recommended.
3. Cover the plate with the well plate sealer and incubate at 37°C for 2 hours.
4. Remove the plate sealer and discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.
5. Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper Do not allow the wells to completely dry at any time.
6. Add 100 µL of prepared 1X Biotinylated PRDX4 Detector Antibody to each well.
7. Cover with the well-plate sealer and incubate at 37°C for 60 minutes.
8. Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.
9. Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.
10. Wash plate 3 times with 1X Wash Buffer as follows:

    10.1Add 300 µL of 1X Wash Buffer to each assay well.

    10.2 Incubate for 1 minute.

    10.3 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle.

    10.4 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

    10.5 Repeat steps 10.1 through 10.4 two more times.
11. Add 100 µL of prepared 1X Avidin-HRP Conjugate into each well, cover with plate sealer and incubate at 37°C for 60 minutes.
12. Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.
13. Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.
14. Wash plate 5 times with 1X Wash Buffer as in Step 10.
15. Add 90 µL of TMB Substrate to each well, cover with plate sealer and incubate at 37°C in the dark for 15-30 minutes. Wells should change to gradations of blue. If the color is too deep, reduce the incubation time. (NOTE: optimal incubation time must be determined by the user. Optimal development can be visualized by blue shading in the top four standard wells, while the remaining standards are still clear.)
16. Add 50 µL of Stop Solution to each well. Well color should change to yellow immediately. Add the Stop

Solution in the same well order as done for the TMB Substrate.

17. Read the O.D. absorbance at 450 nm with a standard microplate reader within 5 minutes of stopping the reaction in step 16. If wavelength correction is available, set to 540 nm or 570 nm.

Data Analysis

Calculation of Results

For analysis of the assay results, calculate the Relative OD_{450} for each test or standard well as follows:

(Relative OD_{450}) = (Well OD_{450}) – (Mean Blank Well OD_{450})

The standard curve is generated by plotting the mean replicate Relative OD_{450} of each standard serial dilution point vs. the respective standard concentration. The concentration contained in the samples can be interpolated by using linear regression of each mean sample Relative OD_{450} against the standard curve. This is best achieved using curve fitting software. A standard curve should be generated each time the test is performed.

Note: if wavelength correction readings were available, subtract the readings at 540 nm or 570 nm from the readings at 450 nm. This may provide greater reading accuracy.

Note: if the samples measured were diluted, multiply the derived mean sample concentration by the dilution factor for a final sample concentration.

Assay Summary

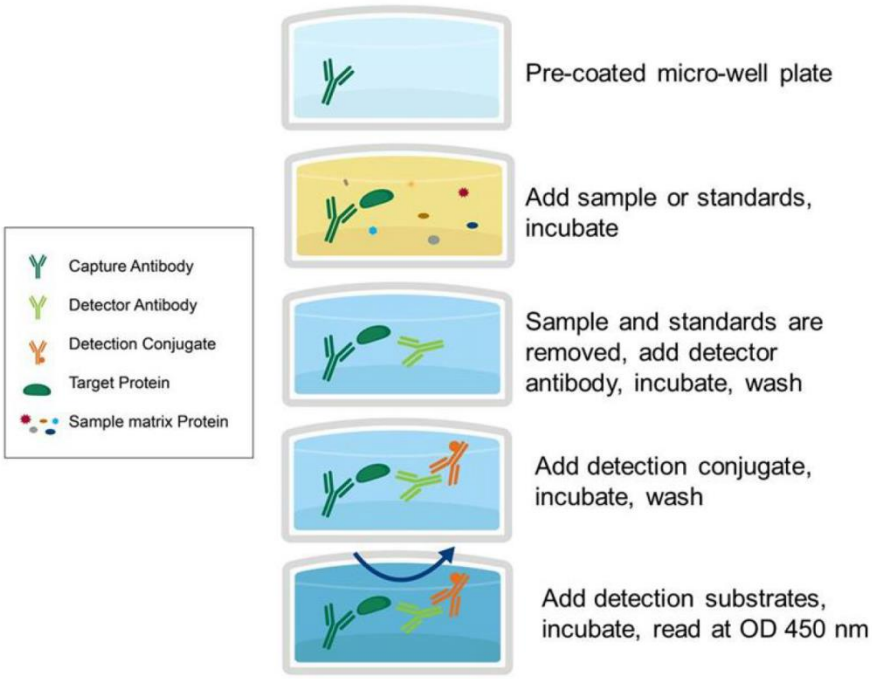

Plate Layout

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