Version
1.1

Human APE1/Ref-1 ELISA Kit

User Manual

Cat.no MR-APE064
MR-APE065

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Please read the entire manual carefully before starting your experiment

This kit is for research use only

MediRedox

1. Introduction

The quantitative enzyme-linked immunosorbent assay (ELISA) kit by MediRedox's is designed for quantitative measurement of APE1/Ref-1 protein in human and murine body fluids and in cell culture supernatant. This kit is based on a sandwich assay principle and can be used to measure the concentration of APE1/Ref-1.

2. Supplied materials and storage

Refer to the list of supplied materials for storage conditions of individual components. Observe the storage conditions for individual prepared components provided in the section Reagent and Standard Preparation.

Human APE1/Ref-1 ELISA Kit materials

Materials	volume	Amount	storage
Pre-coated APE1/Ref-1 ELISA Plate	96well	1 plate	2-8℃
APE1/Ref-1 Standard protein (0.01 ㎎/㎖)	50 ㎕	3 vials	-20℃
200X APE1/Ref-1 detector antibody	60 ㎕	1 vial	-20℃
200X Anti-HRP conjugated antibody	60 ㎕	1 vial	-20℃
10X wash buffer	50 ㎖	1 bottle	-20℃
Diluent buffer	50 ㎖	1 bottle	-20℃
TMB solution	15 ㎖	1 bottle	2-8℃
Stop solution	15 ㎖	1 bottle	2-8℃

Note:

Upon receipt the kit should be stored at 4°C if intended for use within 24 hours. Otherwise the reagents APE1/Ref-1 Standard protein, 200X detector antibody, 200X Anti-HRP conjugated antibody, 10X wash buffer, and diluent buffer should be stored at -20°C. Avoid repeated freeze-thaw cycles. Store all other kit components at 4°C.
The Substrate should never be frozen. Once individual reagents are opened, it is recommended that the kit be used within one month. Unused Strip Plate wells should be stored at 4°C in a sealed bag containing desiccant in order to minimize exposure to moisture. Do not use the kit beyond its expiration date.

3. Additional materials required

The following materials are not included in the kit and need to be supplied by the user.
Microplate reader capable of measuring absorbance at 450nm

① Precision pipettes
② Disposable tips
③ Multichannel pipette
④ Adjustable 1-25ml-pipettes to prepare reagents
⑤ Tubes to prepare standards or sample dilutions
⑥ Reservoirs
⑦ Incubator for incubation at 37°C
⑧ Deionized water
⑨ Absorbent paper

4. Sample collection

MediRedox recommends to use that samples immediately upon preparation. Alternatively, samples stored at 2-8°C should be used within five days. For long-term storage, sample aliquots should be prepared and stored at -20°C if used within one month, or at -80°C if used within six months. Long-term storage can result in protein degradation and denaturation, which may produce inaccurate results. Avoid repeated freeze/thaw cycles of all type of samples.

This assay is designed for use with samples such as plasma or urine. The sample collection protocols below are provided for your reference.

1) **Plasma:** collect plasma using heparin or EDTA as an anticoagulant. Centrifuge the samples for 15 minutes at 3000rpm and 2-8°C. Collect the supernatant for use in the assay.

2) **Urine:** Collect urine using tubes containing a protease inhibitor. Centrifuge the samples for 15 minutes at 12000rpm and 2-8°C. Collect the supernatant for use in the assay.

3) **Cell culture supernatants:** centrifuge the samples for 3 minutes at 1200rpm to remove particulates. Collect the supernatant for use in the assay.

5. Reagent preparation

① All reagents and samples should be at room temperature (18-25°C) before use.

② 10X wash buffer should be diluted with deionized water before use.

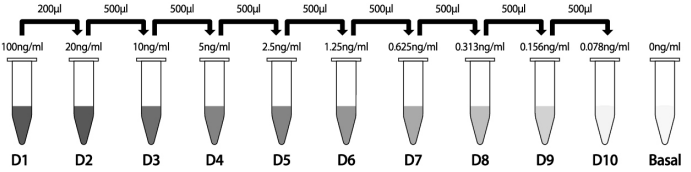
③ Sample dilution: samples should be diluted using the diluent(s)

Note: concentration of target protein may vary by orders of magnitude between different samples, thus the optimal dilution factors for each sample must be determined by the user.

④ Preparation of a standard: appropriate standard preparation will be determined during the development process.

6. Standard Preparation

The following instructions are for the preparing a standard dilution series which will be used to generate a standard curve. The standard curve is required to determine the concentration of target antigen in unknown samples. Volume provided in the following instructions are sufficient perform the standard dilution series in duplicate. APE1/Ref-1 standard and prepared standard dilutions should be used immediately and should not be stored.



Standard Stock solution : 0.01mg/ml recombinant protein

D1 (100ng/ml) : 990µl of diluent into 10µl of 0.01mg/ml protein
D2 (20ng/ml) : 800µl of diluent into 200µl of D1
D3 (10ng/ml) : pipette 500µl of D2 into 500µl of diluent
D4 (5ng/ml) : pipette 500µl of D3 into 500µl of diluent
D5 (2.5ng/ml) : pipette 500µl of D4 into 500µl of diluent
D6 (1.25ng/ml) : pipette 500µl of D5 into 500µl of diluent
D7 (0.625ng/ml) : pipette 500µl of D6 into 500µl of diluent
D8 (0.313ng/ml) : pipette 500µl of D7 into 500µl of diluent
D9 (0.156ng/ml) : pipette 500µl of D8 into 500µl of diluent
D10 (0.078ng/ml) : pipette 500µl of D9 into 500µl of diluent.
Basal (0ng/ml) : only diluent

7. Assay procedure(20~0.313ng/ml)

① Prepare 20ng/ml of APE1/Ref-1 in diluent buffer and perform the serial dilution (D2 – D8) in duplicate.
*Refer to section“6. Standard preparation” for details

② Add 100µl of the prepared standard protein (D2 - D8), basal (diluent only), and sample to each well. Incubate at 37°C for 90 minutes.

③ Discard the liquid and wash the wells five times using wash buffer*. Wash by aspirating or decanting from wells then dispensing 250µl wash buffer into each well. After the last wash invert the plate and blot it against clean paper towels to remove excess liquid.
*10X wash buffer must be diluted with deionized water before use.

④ Dilute 200x APE1/Ref-1 detector antibody in diluent buffer, and add 100µl diluted detection antibody to each well. Incubate at 37°C for 2 hours.

⑤ Discard diluted detection antibody, and wash the wells seven times using wash buffer. Wash by aspirating or decanting from wells then dispensing 250µl wash buffer into each well. After the last wash invert the plate and blot it against clean paper towels to remove excess liquid.

⑥ Dilute 200x anti-HRP conjugated antibody in diluent buffer, and add 100µl diluted anti-HRP conjugated antibody to each well. Incubate at room temperature for 30 minutes.

⑦ Discard diluted anti-HRP conjugated antibody, and wash the wells five times using wash buffer. Wash by aspirating or decanting from wells then dispensing 250µl wash buffer into each well. After the last wash invert the plate and blot it against clean paper towels to remove excess liquid.

⑧ Add 100µl of TMB substrate to each well.

⑨ Incubate at room temperature for 10 minutes (absorbance increases with blue color intensity).

⑩ Add 100µl of Stop Solution to each well, and measure absorbance at 450nm immediately. (When the Stop Solution is added, absorbance increases with yellow color intensity).

⑪ Use the standard curve to quantify APE1/Ref-1 protein concentrations.

7.1 Assay procedure (5~0.078ng/ml)

① Prepare 5ng/ml of APE1/Ref-1 in diluent buffer and perform the serial dilution (D4–D10) in duplicate.
*Refer to section “6. Standard preparation” for details

② Add 100µl of the prepared standard protein (D4– D10), basal (diluent only), and sample to each well. Incubate at 37°C for 90 minutes.

③ Discard the liquid and wash the wells five times using wash buffer*. Wash by aspirating or decanting from wells then dispensing 250µl wash buffer into each well. After the last wash invert the plate and blot it against clean paper towels to remove excess liquid.

*10X wash buffer must be diluted with deionized water before use.

④ Dilute 200x APE1/Ref-1 detector antibody in diluent buffer, and add 100µl diluted detection antibody to each well. Incubate at 37°C for 2 hours.

⑤ Discard diluted detection antibody, and wash the wells seven times using wash buffer. Wash by aspirating or decanting from wells then dispensing 250µl wash buffer into each well. After the last wash invert the plate and blot it against clean paper towels to remove excess liquid.

⑥ Dilute 200x anti-HRP conjugated antibody in diluent buffer, and add 100µl diluted anti-HRP conjugated antibody to each well. Incubate at room temperature for 30 minutes.

⑦ Discard diluted anti-HRP conjugated antibody, and wash the wells five times using wash buffer. Wash by aspirating or decanting from wells then dispensing 250µl wash buffer into each well. After the last wash invert the plate and blot it against clean paper towels to remove excess liquid.

⑧ Add 100µl of TMB substrate to each well.

⑨ Incubate at room temperature for 10 minutes (absorbance increases with blue color intensity).

⑩ Add 100µl of Stop Solution to each well, and measure absorbance at 450nm immediately. (When the Stop Solution is added, absorbance increases with yellow color intensity).

⑪ Use the standard curve to quantify APE1/Ref-1 protein concentrations.

8. Assay procedure note

① ELISA plate: keep appropriate numbers of strips for one experiment and remove extra strips from microplate. Place removed strips should be placed in a sealed bag containing desiccant, and store at 4°C.

② Solutions: to avoid cross-contamination, change pipette tips between additions of each standards, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

③ Applying solutions: solutions should always be added to the bottom of the ELISA plate well. Avoid touching the inside wall of the well. Avoid producing foam when possible.

④ Assay timing: the intervals between adding sample to the first and to the last wells should be minimal. Delays will increase the difference in incubation time between wells, which will significantly affect the experimental accuracy and repeatability. For each step in the procedure, total dispensing time for addition of reagents or samples should not exceed 10 minutes.

⑤ Incubation: do not allow wells to dry out at any time during the assay. Strictly adhere to indicated incubation times and temperatures.

⑥ Washing: proper washing procedure is critical. Insufficient washing will result in poor precision and inaccurately increased absorbance. Residual liquid in the reaction wells should be patted dry using absorbent paper during the washing process. Do not insert absorbent paper into the reaction wells.

⑦ Controlling substrate reaction time: after the adding the TMB substrate, periodically monitor the color development. Stop the reaction before the color turn too deep by adding Stop Solution. Excessively color intensity will produce inaccurate absorbance readings.

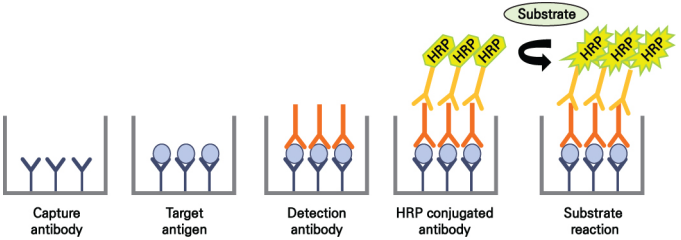
⑧ Reading: the microplate reader should be programmed before use. Prior to taking OD readings, remove any residual liquid or fingerprints from the bottom of the plate, and confirm that the wells do not contain any bubbles.

⑨ Reaction time control: the indicated reaction times should be strictly adhered to.

⑩ Stop Solution: the Stop Solution contains an acid, therefore adequate precautions should be taken during its use, such as protection of the eyes, hands, face, and clothing.

⑪ To minimize external influence on the assay performance, operational procedures and lab conditions (such as room temperature, humidity, or incubator temperature) should be strictly controlled. It is also strongly suggested that the entire assay is conducted by one single user throughout.

9. Assay procedure summary



① Prepare all reagents, samples and standards as indicated.

② Add 100µl standard, basal (diluent only), and sample to each well. Incubate at 37°C for 90 minutes.

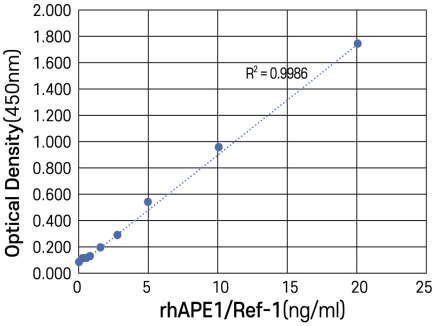
③ Discard the liquid and wash the wells five times.

④ Add 100µl of diluted detection antibody to each well. Incubate at 37°C for 120 minutes.
⑤ Discard the liquid and wash the wells seven times.
⑥ Add 100µl of diluted anti-HRP conjugated antibody to each well. Incubate at room temperature for 30 minutes.
⑦ Discard the liquid and wash the wells five times.
⑧ Add 100µl of TMB substrate to each well. Incubate at room temperature for 10 minutes.
⑨ Add 100µl of Stop Solution to each well. Immediately measure absorbance at 450nm.

10. Calculation of results

Average the duplicate readings for each standard, basal, and sample, and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a four- parameter logistic (4-PL) curve fit. Alternatively, produce standard curve by plotting the mean absorbance of each standard on the x-axis and the concentration on the y-axis, and draw a best-fit curve using the points on the graph. The data may be linearized by plotting a log-transformation of the target antigen concentrations versus the log-transformation optical density; the best-fit line can then be determined by a regression analysis. If samples were diluted, the concentration result according to the standard curve must be multiplied by the respective dilution factor.

(ng/ml)	Average	Average -Basal
20	1.706	1.633
10	0.955	0.882
5	0.518	0.445
2.5	0.298	0.225
1.25	0.199	0.126
0.625	0.134	0.061
0.313	0.115	0.042
0 (Basal)	0.073	0



Typical data: the following standard curve is an example only and should not be used to calculate results for assayed samples. A new standard curve must be generated for each set of assayed samples.

11. Troubleshooting guide

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standard dilution	Briefly spin the vial containing the standard, and mix the liquid gently but thoroughly
Low signal	Too short incubation times	Ensure sufficient incubation time
	Incorrect assay temperature	Use indicated incubation temperature. Bring substrate to room temperature before use
	Inadequate reagent volumes or Improper dilution	Check pipettes and ensure correct preparation
Large CV	Inaccurate pipetting	Check pipettes
	Air bubbles in wells	Remove bubbles from wells
High back ground	Concentration of detector too high	Use indicated dilution factor
	Plate is insufficiently washed	Review the manual for proper washing instructions. If using a plate washer, check that all ports are permeable
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	All the reagents should be stored according to the instructions
	No stop solution added	Add stop solution to each well before reading plate

12. Precautions and support

Usage: this kit is for research use only.

Warning: This reagent may contain sodium azide and sulfuric acid. Chemical, physical, and toxicological properties of these materials have not been thoroughly investigated. Standard Laboratory Practices should be followed. Avoid skin and eye contact, inhalation and ingestion. Sodium azide forms hydrazoic acid under acidic conditions and may react with lead or copper plumbing by forming highly explosive metal azides. On disposal, flush with large volumes of water to prevent accumulation.

Technical Support: MediRedox's professional staff scientists are available to answer any questions about this kit. Email send to your detailed questions to master@mediredox.com.

