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User's Manual

Horse PAI1 (Plasminogen activator inhibitor 1) ELISA Kit

REF **DEIA-FN1360**

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

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

For quantitative detection of Horse PAI1 (Plasminogen activator inhibitor 1) in serum, plasma, tissue homogenates and other biological fluids.

Principles of Testing

This ELISA kit uses Competitive-ELISA as the method. The microtiter plate provided in this kit has been pre-coated with SERPINE1. During the reaction, SERPINE1 in the sample or standard competes with a fixed amount of SERPINE1 on the solid phase supporter for sites on the Biotinylated Detection Antibody specific to SERPINE1. Excess conjugate and unbound sample or standard are washed from the plate, and HRP-Streptavidin is added to each microplate well and incubated. TMB is then used to visualize HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue color product that changes into yellow after adding acidic stop solution. The density of yellow coloration is inversely proportional to the amount of Corticosterone captured in plate.

Reagents And Materials Provided

1. 96-well strip plate (Dismountable), 1 plate
2. Lyophilized Standard, 2 vials
3. Sample/Standard dilution buffer, 20 mL
4. Biotin-detection antibody (Concentrated), 60 uL
5. Antibody dilution buffer, 10 mL
6. HRP-Streptavidin Conjugate(SABC), 120 uL
7. SABC dilution buffer, 10 mL
8. TMB substrate, 10 mL
9. Stop solution, 10 mL
10. Wash buffer (25X), 30 mL
11. Plate Sealer, 5 pieces
12. Product Manual, 1 copy

Materials Required But Not Supplied

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

- 1 Microplate reader capable of measuring absorbance at 450 nm.

- 37°C incubator.

- Precision pipettes to deliver 2 µL to 1 mL volumes.

- Adjustable 1-25 mL pipettes for reagent preparation.

- Distilled or deionized water.

- Log-log graph paper or computer and software for ELISA data analysis.

- Tubes to prepare standard or sample dilutions.

Storage

Store the unopened product at 2 - 8 °C. Do not use past expiration date.

Specimen Collection And Preparation

Isolate the test samples soon after collecting, then, analyze immediately (within 2 hours). Or aliquot and store at -20°C for long term. Avoid multiple freeze-thaw cycles.

Serum: Allow samples to clot for 2 hours at room temperature or overnight at 4°C before centrifugation for 20 minutes at approximately 1000×g. Collect the supernatant and carry out the assay immediately. Blood collection tubes should be disposable, non-pyrogenic, and non-endotoxin.

Plasma: Collect plasma using EDTA-Na2 as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2 - 8°C within 30 minutes of collection. Collect the supernatant and carry out the assay immediately. Avoid hemolysis, high cholesterol samples.

Tissue homogenates: For general information, hemolysis blood may affect the result, so you should rinse the tissues with ice-cold PBS (0.01 M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then minced to small pieces which will be homogenized in PBS (the volume depends on the weight of the tissue. 9 mL PBS would be appropriate to 1 gram tissue pieces. Some protease inhibitor is recommended to add into the PBS.) with a glass homogenizer on ice. To further break the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifugated for 5 minutes at 5000×g to get the supernate.

Cell culture supernate: Centrifuge supernate for 20 minutes to remove insoluble impurity and cell debris at 1000×g at 2 - 8°C. Collect the clear supernate and carry out the assay immediately.

Other biological fluids: Centrifuge samples for 20 minutes at 1000×g at 2 - 8°C. Collect the supernatant and carry out the assay immediately.

Sample preparation: Samples should be clear and transparent and be centrifuged to remove suspended solids.

Note: Samples to be used within 5 days may be stored at 4°C, otherwise samples must be stored at -20°C (≤1 month) or -80°C (≤2 months) to avoid loss of bioactivity and contamination. Hemolyzed samples are not suitable for use in this assay.

Reagent Preparation

Bring all kit components and samples to room temperature (20 - 25°C) before use.

1.Samples

Please predict the concentration before assaying. If concentrations are unknown or not within the detection range, a preliminary experiment is recommended to determine the optimal dilution.

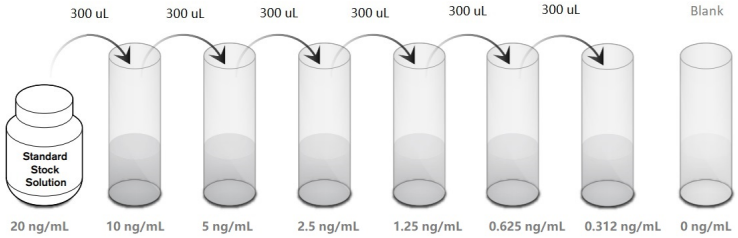
2. Wash Solution

If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 30 mL of Wash Solution concentrate (25×) with 720 mL of deionized or distilled water to prepare 750 mL of Wash Solution (1×).

3. Standard

Add 1 ml of Sample / Standard dilution buffer into one Standard tube, This reconstitution produces a stock solution of 20 ng/mL. Allow the standard to sit for a minimum of 10 minutes with gentle agitation prior to making dilutions.

Pipette 300 µL of the Sample / Standard dilution buffer into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer.



Note: The standard solutions are best used within 2 hours.

4. Preparation of Biotin-detection Antibody working solution

Dilute the Biotin-detection antibody with Antibody dilution buffer at 1:100 and mix thoroughly within 1 hour of use. (i.e. Add 1 µL of Biotin-detection antibody into 99 µL of Antibody dilution buffer.)

5. Preparation of HRP-Streptavidin Conjugate (SABC) working solution

Dilute the SABC with SABC dilution buffer at 1:100 and mix thoroughly within 30 minutes of use. (i.e. Add 1 µ L of SABC into 99 µL of SABC dilution buffer.)

Assay Procedure

Bring all reagents and samples to room temperature before use. It is recommended that all standards, samples, and controls be assayed in duplicate.

1. Set standard, test sample and control (zero) wells on the pre-coated plate respectively, and then, record their positions. It is recommend to measure each standard and sample in duplicate. **Wash plate 2 times before adding standard, sample and control (zero) wells!**
2. **Add Sample and Biotin-detection antibody:** Add 50 µL of Standard, Blank, or Sample per well. The blank well is added with Sample / Standard dilution buffer. Immediately add 50 µL of Biotin-detection antibody working solution to each well. Cover with the plate sealer we provided. Gently tap the plate to ensure thorough mixing. Incubate for 45 minutes at 37 °C. (Solutions are added to the bottom of micro ELISA plate well, avoids inside wall touching and foaming to the best of your ability.)
3. **Wash:** Aspirate each well and wash, repeating the process three times Wash by filling each well with Wash Buffer (approximately 350 µL) using a squirt bottle, multi-channel pipette, manifold dispenser or automated washer. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and pat it against thick clean absorbent paper.

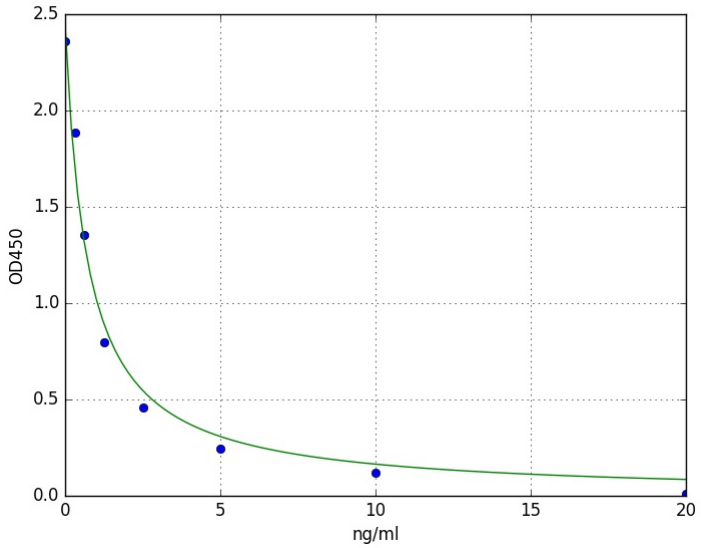
4. **HRP-Streptavidin Conjugate (SABC):** Add 100 µL of SABC working solution to each well. Cover with a new Plate sealer. Incubate for 30 minutes at 37 °C.
5. **Wash:** Repeat the aspiration/wash process for five times.
6. **TMB Substrate:** Add 90 µL of TMB Substrate to each well. Cover with a new Plate sealer. Incubate for about 15-20 minutes at 37 °C. Protect from light. The reaction time can be shortened or extended according to the actual color change, but not more than 30 minutes. When apparent gradient appeared in standard wells, you can terminate the reaction.
7. **Stop:** Add 50 µL of Stop Solution to each well. Color turn to yellow immediately. The adding order of stop solution should be as the same as the substrate solution.
8. **OD Measurement:** Determine the optical density (OD Value) of each well at once, using a microplate reader set to 450 nm. You should open the microplate reader ahead, preheat the instrument, and set the testing parameters.

Calculation

Average the duplicate readings for each standard and samples. Create a standard curve by plotting the mean OD Value for each standard on the y-axis or x-axis against the concentration on the x-axis or y-axis and draw a best fit curve through the points on the graph. It is recommended to use some professional software to do this calculation, such as curve expert 1.3 or 1.4. In the software interface, a best fitting equation of standard curve will be calculated using OD Value and concentrations of standard sample. The software will calculate the concentration of samples after entering the OD Value of samples. Also, you can enter the corresponding fitting equation and OD Value of samples into Excel to get the concentration of samples.

If samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor.

Typical Standard Curve



Precision

Intra-Assay: CV<8%

Inter-Assay: CV<10%

Detection Range

0.312-20 ng/mL

Sensitivity

0.188 ng/mL

Specificity

This assay has high sensitivity and excellent specificity for detection of SERPINE1 . No significant cross-reactivity or interference between Pola2 and analogues was observed.

Note: Limited by current skills and knowledge, it is impossible for us to complete the cross- reactivity detection between SERPINE1 and all the analogues, therefore, cross reaction may still exist.

Linearity

The linearity of the kit was assayed by testing samples spiked with appropriate concentration of SERPINE1 and their serial dilutions. The results were demonstrated by percentage of calculated concentration to the expectation.

Sample	1:2	1:4	1:8	1:16
serum(n=5)	90-99%	91-105%	91-103%	85-105%
EDTA plasma(n=5)	82-101%	95-98%	84-97%	89-98%
heparin plasma(n=5)	83-100%	80-98%	82-99%	81-90%

Recovery

Matrices listed below were spiked with certain level of SERPINE1 and the recovery rates were calculated by comparing the measured value to the expected amount of SERPINE1 in samples.

Matrix	Recovery range(%)	Average(%)
serum(n=5)	86-101	95
EDTA plasma(n=5)	87-105	94
heparin plasma(n=5)	89-105	95

Precautions

1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.
2. Substrate Solution should remain colourless until added to the plate. Keep Substrate Solution protected from light
3. Stop Solution should remain colourless until added to the plate. The colour developed in the wells will turn from blue to yellow immediately after the addition of the Stop Solution. Wells that are green in colour indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.
4. Avoid any contamination among samples and reagents. For this purpose, disposable tips should be used for each sample and reagent
5. Reagents from different batches should not be mixed.
6. Use thoroughly clean glassware
7. We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
8. All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.