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Human Plasmin / Antiplasmin Complex ELISA Kit

Catalog No: CKH021A **Size:** 1 x 96 wells
CKH021B **Size:** 5 x 96 wells

Introduction:

Human Plasmin / Antiplasmin (PAP) Complex ELISA assay is intended for the quantitative determination of the covalent complex of plasmin and its inhibitor α2-antiplasmin in human plasma, serum, urine, cell culture supernatants, and tissue extracts. For research use only.

Plasminogen is a single chain glycoprotein zymogen and is the precursor of the fibrinolytic enzyme plasmin. The serine protease plasmin rapidly forms a 1:1 covalent complex with its major circulating inhibitor α2-antiplasmin. Detection of PAP complex in plasma represents activation of the fibrinolytic system either directly or secondarily to coagulation in vivo.

Human PAP complex in samples will bind to the antihuman plasmin capture antibody coated on the microtiter plate. After appropriate washing steps, polyclonal anti-α2-antiplasmin primary antibody binds to PAP complex captured on the plate. Excess antibody is washed away and bound polyclonal antibody is then reacted with horseradish peroxidase conjugated streptavidin. TMB substrate is used for color development at 450 nm. A standard calibration curve is prepared along with the samples to be measured using dilutions of PAP complex. Color development is proportional to the concentration of PAP complex in the samples. Free plasminogen and antiplasmin will not be detected by this assay.

Reagents Included for 1 x 96 Wells:

Items	Quantity
A: Microtiter plate coated with Anti-Human Plasmin Capture Antibody (blocked and dried)	96 wells (8 x 12-well strips)
B: Wash Buffer Concentrate (10x)	1 bottle (50 ml)
C: Human Plasmin-Antiplasmin Complex Standard (lyophilized)	1 vial
D: Biotinylated Anti-α2-Antiplasmin Detection pAb	1 vial
E: Streptavidin-HRP	1 vial
F: TMB Substrate Solution*	1 bottle (10 ml)

Avoid skin and eye contact when using TMB substrate solution since it may be irritating to eyes, skin, and respiratory system. Wear safety goggles and gloves.

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Storage of Kit Reagents:

All kit components must be stored at 4°C. Store unopened plate and any unused microtiter strips in the pouch with desiccant. Reconstituted standards and primary antibody may be stored at -80°C for later use. **DO NOT freeze/thaw the standards and primary antibody more than once**. All other unused kit components must be stored at 4°C. Kit should be used no later than the expiration date.

Materials/Reagents required but not provided:

- 1-channel pipettes covering 1-10 µl and 200-1000 µl
- 12-channel pipette for 50-500 µl
- Pipette tips
- Paper towels or Kimwipes
- Polypropylene tubes for dilution of standard
- Deionized or distilled water
- Bovine Serum Albumin Fraction V (BSA)
- Tris(hydroxymethyl)aminomethane (Tris)
- Sodium Chloride (NaCl)
- 1 N H_2SO_4
- Manifold dispenser /aspirator or automated microplate washer
- Microtiter plate shaker with uniform horizontally circular movement up to 300 rpm
- Microtiter plate spectrophotometer operable at 450 nm

Precautions:

- **DO NOT** mix any reagents or components of this kit with any reagents or components of any other kit. This kit is designed to work properly as provided.
- Always pour substrate out of the bottle into a clean test tube. **DO NOT** pipet out of the bottle, as you could contaminate the substrate.
- Keep plate covered except when adding reagents, washing, or reading.
- **DO NOT** pipette reagents by mouth and avoid contact of reagents and specimens with skin.
- **DO NOT** smoke, drink or eat in areas where specimens or reagents are being handled.

Preparation of Buffers, Specimen, and Standard:

TBS Buffer

0.1 M Tris + 0.15 M NaCl, pH 7.4

1X Wash Buffer

Dilute 50 ml of 10X wash buffer concentrate with 450 ml of deionized water.

Blocking Buffer (BB)

3% BSA (w/v) in TBS Buffer

Specimen Collection

Collect plasma in sodium citrate, EDTA, or heparin collection tubes. Immediately after collection, centrifuge at 1,000 x g for 15 minutes. Assay immediately or aliquot and store at ≤ 20°C. Avoid repeated freeze-thaw cycles.

Preparation of Standard

Reconstitute standard by adding 10 ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. This will result in a 1,000 ng/mL standard solution.

Table 1: Dilution table for preparation of Human PAP Complex Standard:

PAP Complex Concentration (ng/ml)	Dilutions
1,000	500 µl (from vial)
500	500 µl (BB) + 500 µl (1,000 ng/ml)
200	600 µl (BB) + 400 µl (500 ng/ml)
100	500 µl (BB) + 500 µl (200 ng/ml)
50	500 µl (BB) + 500 µl (100 ng/ml)
20	600 µl (BB) + 400 µl (50 ng/ml)
10	500 µl (BB) + 500 µl (20 ng/ml)
5	500 µl (BB) + 500 µl (10 ng/ml)
2	600 µl (BB) + 400 µl (5 ng/ml)
1	500 µl (BB) + 500 µl (2 ng/ml)
0	500 µl (BB) Zero point to determine background

NOTE: Dilutions for the standard curve must be made and applied to the plate immediately.

ELISA Method:

Be sure to read 'Preparation of Buffers, Specimen, and Standard' before carrying out the assay.

Perform assay at room temperature.

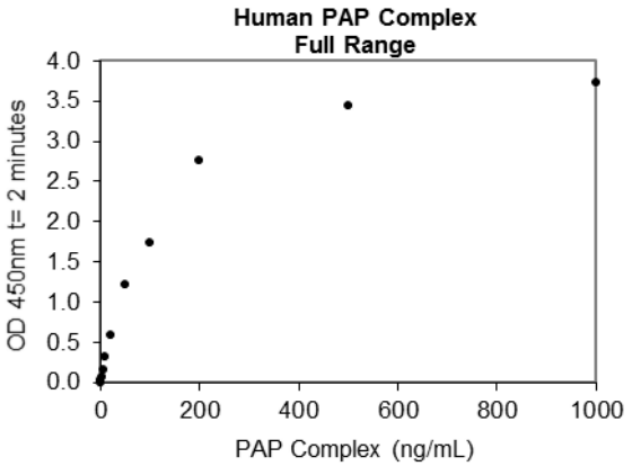
1. Remove microtiter plate from bag. Add 100 µl Plasmin-Antiplasmin Complex Standards in duplicate and unknowns to wells. Carefully record position of standards and unknowns.
2. Shake the plate at 300 rpm for 30 minutes.
3. Wash the wells 3 times with 300 µl Wash Buffer. Remove excess wash by gently tapping plate on paper towel or Kimwipe.
   NOTE: The assay measures PAP complex in the 1-1,000 ng/ml range. If the unknown is thought to have high PAP complex levels, dilutions may be made in blocking buffer. Plasma samples should be applied directly to the plate without dilution.
4. Reconstitute Biotinylated Anti-Human α2-Antiplasmin Polyclonal Detection Antibody by adding 10 ml of Blocking Buffer directly to the vial and agitate gently to completely dissolve contents.
5. Add 100 µl of reconstituted to all wells.
6. Shake plate at 300 rpm for 30 minutes.
7. Wash wells 3X with 300 µl Wash Buffer. Remove excess wash by gently tapping plate on paper towel or Kimwipe.
8. Briefly centrifuge Streptavidin-HRP vial before opening. Dilute 2.5 µl of Streptavidin-HRP into 2.5 ml blocking buffer to generate a 1:1,000 dilution.
9. Add 0.4 ml of 1:1,000 dilution to 9.6 ml of blocking buffer to generate a 1:25,000 dilution. Add 100 µl of the 1:25,000 dilution to all wells.
10. Shake plate at 300 rpm for 30 minutes.

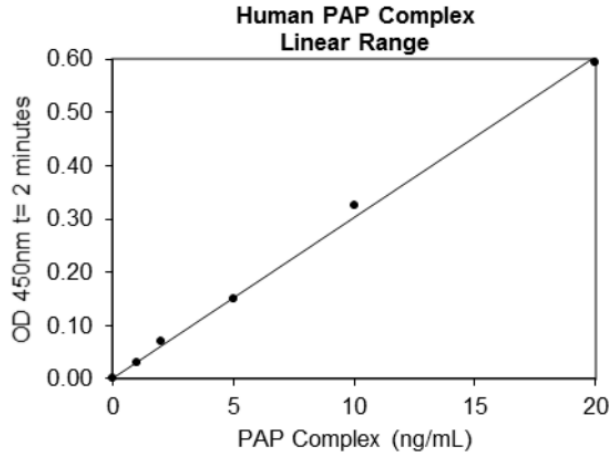
11. Wash wells three times with 300 µl wash buffer. Remove excess wash by gently tapping plate on paper towel or Kimwipe.
12. Add 100 µl of TMB substrate to all wells and shake plate for 2-5 minutes. Substrate will change from colorless to different strengths of blue.
13. Quench the reaction by adding 50 µl of 1 N H_2SO_4 stop solution to all wells when samples are visually in the same range as the standards. Add stop solution to wells in the same order as substrate upon which color will change from blue to yellow. Mix thoroughly by gently shaking the plate. Read final absorbance at 450 nm. For best results read plate immediately.
14. Set the absorbance at 450 nm in a microtiter plate spectrophotometer and measure the absorbance in all wells at 450 nm. Subtract zero point from all standards and unknowns to determine corrected absorbance (A_{450}).

Calculation of Results:

Plot A_{450} against the amount of PAP Complex in the standards. Fit a straight line through the linear points of the standard curve using a linear fit procedure if unknowns appear on the linear portion of the standard curve. Alternatively, create a standard curve by analyzing the data using a software program capable of generating a four parameter logistic (4PL) curve fit. The amount of PAP Complex in the unknowns can be determined from this curve. If samples have been diluted, the calculated concentration must be multiplied by the dilution factor.

A typical standard curve (For example only):





Expected Values:

Concentration of PAP complex in normal human plasma was found to be 0.63 ± 0.05 µg/ml and increased with age.

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Performance Characteristics:

Sensitivity: The minimum detectable dose (MDD) was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates (range OD450: 0.052 - 0.06) and calculating the corresponding concentration. The MDD was 0.155 ng/ml.

Sample Values: Samples were evaluated for the presence of antigen at varying dilutions.

Sample Type	Dilution	Mean (ng/ml)
Citrate Plasma	Undiluted	2.9

NOT FOR HUMAN USE. FOR RESEARCH ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

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