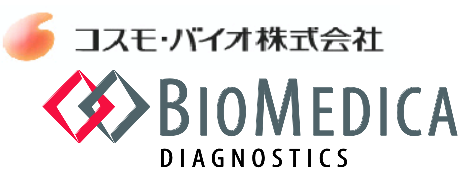

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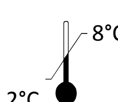
IMUCLONE™ PAP ELISA

For Research Use Only



INTENDED USE

The IMUCLONE™ PAP ELISA is an enzyme-linked sandwich immunoassay for the measurement of plasmin/alpha-2-antiplasmin (PAP) complexes in human plasma. The assay measures only PAP complexes. It neither measures nor is affected by free plasminogen, alpha-2-antiplasmin or other plasmin complexes.

It is intended for research use only and not for diagnostic procedures.

EXPLANATION OF THE TEST

Alpha-2-antiplasmin (α-2-antiplasmin) is a single chain 70 kD plasmin inhibitor which rapidly reacts with plasmin to form the inactive plasmin/alpha-2-antiplasmin complex. Synthesized by the liver, alpha-2-antiplasmin circulates in plasma at a concentration of approximately 1 µM (70 µg/mL), with 20% being cross-linked when blood clots. The formation of the PAP complex is a two-step process. First, the lysine binding sites of plasmin and the carboxyl-terminal region of alpha-2-antiplasmin form a reversible complex. In the second step, cleavage of the peptide bond of the inhibitor forms the irreversible complex.

Alpha-2-antiplasmin is consumed during thrombolytic therapy. Increased PAP complex formation is accompanied by increased fibrin formation and an increased reactive plasminemia. Accordingly, a correlation between the level of fibrin split products and the level of PAP complexes exists.

PRINCIPLE OF THE METHOD

The IMUCLONE PAP ELISA uses a murine monoclonal antibody specific against the neoantigen in the human PAP complex as the capture antibody. Diluted plasma samples incubate in microwells pre-coated with the antibody, extraneous plasma proteins are washed away, and a horseradish peroxidase (HRP) conjugated monoclonal antibody directed against human plasminogen is added, to complete the formation of the antibody/antigen/antibody "sandwich".

The addition of a 3,3',5,5' - tetramethylbenzidine (TMB) substrate, and its subsequent reaction with the HRP creates a blue colored solution. Sensitivity is increased by addition of a sulfuric acid stop solution, yielding a yellow color. PAP complex levels are quantified by measuring solution absorbances at 450 nm and comparing the values with those from a standard curve.

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REAGENTS

96 MAb Anti-Human PAP Complex IgG Coated Microwells
1 vial of Dilution Buffer (20 mL, 2.5x concentrate)
5 vials of PAP Calibrator (lyophilized), see labeled concentration
2 vials of PAP Controls, High/Low (lyophilized), see labeled concentration
1 vial of Detection Antibody, HRP-anti-human plasminogen IgG, 0.3 mL
2 vials of Wash Buffer, 0.15M PBS, 0.05% Tween 20, 20 mL (12.5x concentrate)
1 vial of Substrate, TMB, 12 mL
1 vial of Stop Solution, 0.45 M Sulfuric Acid, 12 mL

WARNINGS AND PRECAUTIONS

Source material for some of the reagents in this kit is of human origin. This material has been found to be non-reactive for Hepatitis B Surface Antigen (HBsAg), Hepatitis C Virus (HCV) and Human Immunodeficiency Virus Type 1 and Type 2 (HIV-1, HIV-2) using FDA approved methods. As no known test method provides complete assurance that products derived from human blood will not transmit HBsAg, HCV, HIV-1, HIV-2 or other blood-borne pathogens, reagents should be handled as recommended for any potentially infectious human specimen. Discard all waste associated with test specimens and human source reagents in a biohazard waste container.

For research use only. Not for internal use in humans or animals. Do not use the kit components beyond the stated expiration date. Do not mix reagents from different kits. Avoid microbial contamination of the reagents. Do not smoke, eat or drink in areas in which specimens or kit reagents are handled. Do not pipette reagents by mouth. Use good laboratory hygiene.

Stop Solution	Warning	⚠	CONT: Sulfuric acid H315, H319, P264, P280, P302 + P352, P305 + P351 + P338, P332 + P313, P337 + P313

Hazard Statements:
H315 Causes skin irritation.
H319 Causes serious eye irritation.

Precautionary Statements:
P264 Wash thoroughly after handling.
P280 Wear protective gloves/ protective clothing/ eye protection/ face protection.
P302 + P352 IF ON SKIN: Wash with plenty of water.
P305 + P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P332 + P313 If skin irritation persists: Get medical advice/attention.
P337 + P313 If eye irritation persists: Get medical advice/attention.

REAGENT PREPARATION AND STORAGE

Unopened and lyophilized reagents are stable until the expiration date printed on the box when stored as instructed.

1. **MAb Anti-Human PAP Complex IgG Coated Microwells**: Once removed from the foil pouch, the microwell strips must be used within 30 minutes. Unused strips may be stored at 2°– 8°C for 4 weeks when sealed in the original pouch with the desiccant present, protected from any moisture.
2. **Dilution Buffer**: Add the 20 mL of concentrated buffer to 30 mL of filtered deionized H_2O. Dilution Buffer may be used for up to 3 months when stored at 2°–8°C.
3. **PAP Calibrators**: Add 0.5 mL of filtered deionized H_2O to each vial and let stand for 15 minutes. Mix well for 10 seconds. Reconstituted Calibrators will be clear to slightly turbid. Calibrators are stable for 4 hours at 18°– 25°C and may be aliquoted and stored at –20°C for up to 6 months.
4. **PAP Controls**: Add 0.5 mL of filtered deionized H_2O to each vial and let stand for 15 minutes. Mix well for 10 seconds. Reconstituted Controls will be clear to slightly turbid. Calibrators are stable for 4 hours at 18°–25°C and may be aliquoted and stored at –20°C for up to 6 months.
5. **Detection Antibody**: Concentrated Detection Antibody is stable until the stated expiration date when properly stored at 2°– 8°C. Prepare working strength Detection Antibody by diluting 1:50 with Dilution Buffer (1 part Detection Antibody + 49 parts Dilution Buffer). Working Strength Detection Antibody is stable for 60 minutes at 18°– 25°C.
6. **Wash Buffer**: Add the 20 mL of concentrated buffer to 230 mL of distilled H_2O. Wash Buffer may be used for up to 3 months when stored at 2°– 8°C.
7. **Substrate**: Supplied ready to use. Substrate is stable until the stated expiration date when properly stored at 2°– 8°C.
8. **Stop Solution**: Supplied ready to use. Stop Solution is stable until the stated expiration date when properly stored at 2°– 8°C.

SPECIMEN COLLECTION AND PREPARATION

1. Plasma samples must be prepared with an "inhibitor cocktail". Blood should be collected in tubes containing 3.2% citrate (1:10 citrate to blood ratio), with aprotinin and benzamidine to final concentrations of 2000 KIU/mL and 20 mM respectively. As an alternative, commercially available collection tubes containing PPACK may also be used.
2. Centrifuge the collected blood at 2,000 x g for 30 minutes at 2 - 8°C.

3. Aliquot and store plasma at -70°C within 90 minutes. Frozen plasma should be thawed rapidly at 37°C.
4. For "Low PAP Levels", dilute patient plasma samples 1:10 in Dilution Buffer (25 µL plasma + 225 µL buffer).

   For "High PAP Levels", dilute patient plasma samples 1:100 in Dilution Buffer (1:1000 for fully activated plasma samples).

PROCEDURE

Materials Provided – See Reagents

Material Required But Not Provided

0.22 µm filtered deionized H_2O, test tubes
50-300 µL eight channel multi-pipette, 0-200 µL, 200-1000 µL single pipettes
1 Liter graduated cylinder
incubator at 37°C
vortexer
microwell plate reader for reading absorbance at 450 nm
microwell plate washer (optional)

Assay Procedure

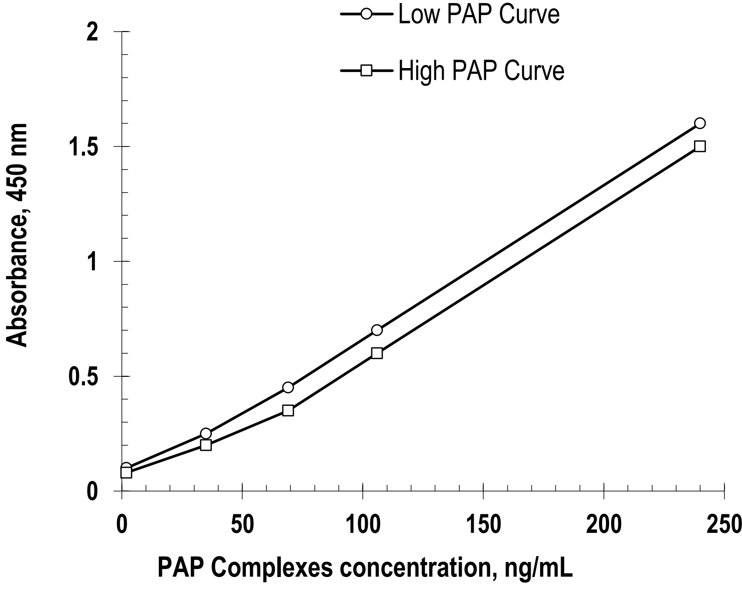
1. Open the foil pouch and remove the frame with the microwell strips. Remove the strips that will not be used and replace in the foil pouch. Tightly reseal the foil pouch and store at 2°-8°C.
2. Wash the microwells 4 times each with 250 µL of Wash Buffer.
3. Add 100 µL of either standard or diluted plasma sample into the microwells, cover the strips with clear plastic foil and incubate overnight at 2°-8°C. Assay standards and samples in duplicate.
4. Wash the microwells 4 times each with 250 µL of Wash Buffer.
5. Add 100 µL of Working Strength Detection Antibody to each microwell, cover and incubate for 2 hours at 37°C. Discard any unused working strength Detection Antibody.
6. Wash the microwells 4 times each with 250 µL of Wash Buffer.
7. Add 100 µL of Substrate solution to each microwell, cover and incubate for 20 minutes at room temperature (20°-25°C).
8. Stop the enzymatic reaction by adding 100 µL of Stop Solution to each microwell. Tap the sides of the microwells to ensure adequate mixing. Read the absorbances on a microwell plate reader at 450 nm within 10 minutes.

RESULTS

The standard curve is constructed by plotting the mean absorbance value calculated for each PAP complex standard versus the corresponding concentration. Interpolate the PAP complex concentration for each diluted sample directly from the standard curve. A standard curve should be generated each time the assay is run.

Representative Standard Curve

The following standard curve is for demonstration purposes only.



CALCULATION OF RESULTS

Multiply the PAP complex concentration for the diluted plasma sample interpolated from the standard curve by the dilution factor to obtain the actual PAP complex concentration of the patient plasma. The calculation is,

[PAP]Plasma Sample = [PAP]Diluted Test Sample x D (Dilution Factor)

LIMITATIONS OF THE PROCEDURE

Plasma may be collected on citrate without inhibitors only if it will not contain any thrombolytic reagents. If the plasma does contain thrombolytic reagents, PAP complex formation may occur and elevated levels will be measured.

PERFORMANCE CHARACTERISTICS

The intra- and inter-assay variations are less than 5% and 10% respectively.

STANDARDIZATION

The Calibrators have been standardized against PAP complexes prepared *in vitro* using purified proteins.

REFERENCES

1. Aoki, N., *et al.* *Seminars in Thrombosis and Haemostasis* 1984, **10**: 24-41.
2. Collen, D. *Thrombosis and Haemostasis* 1980, **43**: 77-89.
3. Holmes, W. E., *et al.* *Journal of Biological Chemistry* 1987, **262**: 1659-1664.
4. Sakata, Y., Aoki, N. *Journal of Clinical Investigations* 1982, **69**: 536-542.
5. Hattey, E., *et al*. Fibrinolysis Suppl. Abstract #152, 1986.
6. Hattey, E., *et al.* *ECAT Assay Procedures; A Manual of Laboratory Techniques.* Kluwer Academic, 1992: 173-193.

DEFINITIONS OF SYMBOLS

Symbol	Definition
	Consult instructions for use
	Manufactured by
LOT	Batch code / Lot number
	Expiration Date
Σ n	Contains sufficient for <n> tests
	Refer to SDS
2°C 8°C	Temperature limitation: Store at 2°C to 8°C
REF	Catalog number
CONT	Contains...

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