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Cynomolgus Monkey Plasminogen Total Antigen ELISA Kit

Catalog No.	CKK000A	**Quantity:**	1 x 96 tests
	CKK000B	**Quantity:**	5 x 96 tests

Intended Use: This cynomolgus macaque (*Macaca fascicularis*) monkey plasminogen total assay is for the quantitative determination of total plasminogen antigen in plasma, serum, or cell culture media. This kit has been formulated for research only.

Background: Plasminogen is a single chain glycoprotein zymogen and is the precursor of the fibrinolytic enzyme plasmin. Plasminogen deficiencies are classified as hypoplasminogenemia (Type I) or dysplasminogenemia (Type 2) and are associated with decreased extracellular fibrin clearance leading to mucous membrane lesions and ligneous conjunctivitis.

Assay Principle: Cynomolgus monkey plasminogen will bind to the affinity purified capture antibody coated on the microtiter plate. Plasminogen, plasmin, and plasmin in complex with antiplasmin will react with the antibody on the plate. After appropriate washing steps, biotin labeled polyclonal anti-cynomolgus plasminogen primary antibody binds to the captured protein. Excess antibody is washed away and bound polyclonal antibody is then reacted with peroxidase conjugated streptavidin. Following an additional washing step, 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 cynomolgus plasminogen. Color development is proportional to the concentration of total plasminogen in the sample.

Reagents Provided:
A: 96-well antibody coated microtiter strip plate (removable strips 8x12): containing anti-cynomolgus plasminogen antibody dried and blocked on the strip well surface
B: 10X Wash Buffer: 1 bottle of 50 ml; bring to 1X using DI water
C: Cynomolgus plasminogen activity standard: 1 vial lyophilized standard
D: Biotinylated anti-plasminogen detection antibody: 1 vial lyophilized anti-cynomolgus polyclonal antibody
E: Horseradish peroxidase streptavidin: 1 vial concentrated HRP labeled streptavidin
F: TMB substrate solution: 1 bottle 10 ml solution

Storage and Stability: 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 may be stored at -80°C for later use. DO NOT freeze/thaw the standard and primary antibody more than once. All other unused kit components must be stored at 4°C. The kit should be used no later than the expiration date.

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Reagents and Equipment Required:

- Microtiter plate shaker capable of 300 rpm uniform horizontally circular movement
- Manifold dispenser/aspirator or automated microplate washer
- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes and Pipette tips
- Deionized or distilled water
- Polypropylene tubes for dilution of standard
- Paper towels or laboratory wipes
- 1N H_2SO_4 or 1N HCl
- Bovine Serum Albumin Fraction V (BSA)
- Tris(hydroxymethyl)aminomethane (Tris)
- Sodium Chloride (NaCl)

Warnings:

Warning – 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.

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.
- **DO NOT** pipette reagents by mouth.
- Always pour substrate out of the bottle into a clean test tube.
- **DO NOT** pipette out of the bottle as you could contaminate the substrate.
- Keep plate covered except when adding reagents, washing, or reading.
- All kit components must be kept refrigerated (4°C).
- **DO NOT** smoke, drink, or eat in areas where specimens or reagents are being handled.

Preparation of Reagents:

TBS buffer: 0.1 M TRIS, 0.15 M NaCl, pH 7.4

Blocking buffer (BB): 3% BSA (w/v) in TBS buffer

1 x Wash buffer: Dilute 50 ml of 10X wash buffer concentrate with 450 ml deionized water.

Specimen Collection:

Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for at least 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store at $\leq$-20°C. Avoid repeated freeze-thaw cycles.

Assay Procedure:

Perform assay at room temperature. Vigorously shake plate (300rpm) at each step of the assay.

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Preparation of Standard:
Reconstitute standard by adding 1 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.

Plasminogen concentration (ng/ml)	Dilutions
100	900µl (BB) + 100µl (from vial)
50	500µl (BB) + 500µl (100ng/ml)
20	600µl (BB) + 400µl (50ng/ml)
10	500µl (BB) + 500µl (20ng/ml)
5	500µl (BB) + 500µl (10ng/ml)
2	600µl (BB) + 400µl (5ng/ml)
1	500µl (BB) + 500µl (2ng/ml)
0.5	500µl (BB) + 500µl (1ng/ml)
0	500µl (BB) Zero point to determine background

NOTE: DILUTIONS FOR THE STANDARD CURVE MUST BE MADE AND APPLIED TO THE PLATE IMMEDIATELY.

Standard and Unknown Addition:
Remove microtiter plate from bag and add 100 µl plasminogen standards (in duplicate) and unknowns to wells. Carefully record position of standards and unknowns. Shake plate at 300 rpm for 30 minutes. Wash wells three times with 300 µl wash buffer. Remove excess wash by gently tapping plate on paper towel or Kimwipe.
NOTE: The assay measures plasminogen and plasmin antigens in the 0.5-100 ng/ml range. If the unknown is thought to have high plasminogen/plasmin levels, dilutions may be made in a similar biological fluid devoid of plasminogen or in blocking buffer. A 1:10,000 - 1:100,000 dilution for normal cynomolgus plasma is suggested for best results.

Detection Antibody Addition:
Reconstitute biotinylated detection antibody by adding 10 ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. Add 100 µl to all wells. Shake plate at 300 rpm for 30 minutes. Wash wells three times with 300 µl wash buffer. Remove excess wash by gently tapping plate on paper towel or Kimwipe.

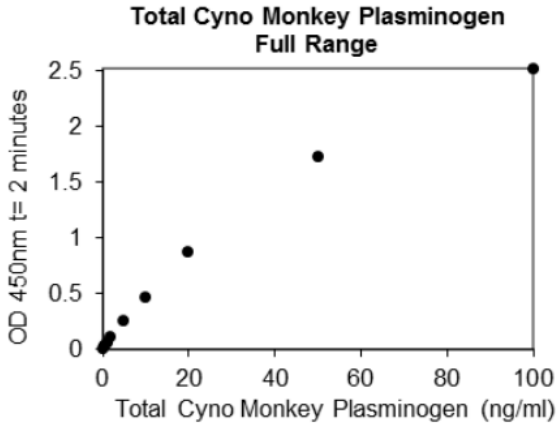
Streptavidin-HRP Addition:
Briefly centrifuge vial before opening. Dilute 2.5 µl of HRP conjugated streptavidin into 2.5 ml blocking buffer to generate a 1:1,000 dilution. Add 0.2 ml of the 1:1,000 dilution to 9.8 ml of blocking buffer to generate a 1:50,000 dilution. Add 100 µl of the 1:50,000 dilution to all wells. Shake plate at 300 rpm for 30 minutes. Wash wells three times with 300 µl wash buffer. Remove excess wash by gently tapping plate on paper towel or Kimwipe.

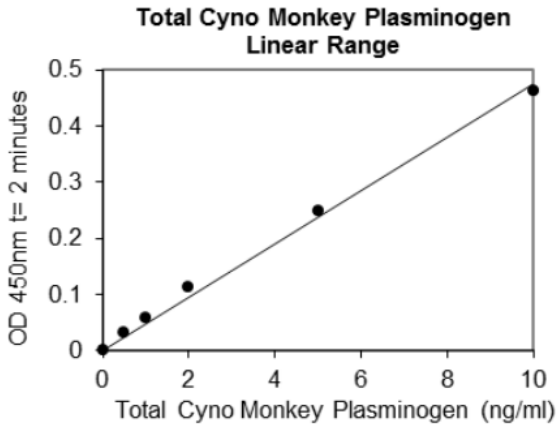
Substrate Incubation:
Add 100 µl TMB substrate to all wells and shake plate for 2-10 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50 µl of 1N H_2SO_4 of HCl stop solution to all wells when samples are visually in the same range as the standards. Add stop solution to wells in the same order as substrate upon which color will change from blue to yellow. Mix thoroughly and read final absorbance values at 450 nm. For best results read plate immediately.

Measurement:
Set the absorbance at 450 nm in a microtiter plate spectrophotometer. 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 plasminogen in the standards. Fit a straight line through the points 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 plasminogen 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.
(EXAMPLE ONLY, DO NOT USE)





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Expected Values: The concentration of plasminogen in normal human pooled donor plasma was found to be 195 ± 10 µg/ml. Normal values of plasminogen in cynomolgus plasma have not been conclusively determined but are believed to be similar to human plasma.

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 OD_{450}: 0.18-0.199057) and calculating the corresponding concentration. The MDD was 0.243 ng/ml.

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

Sample Type	Dilution	Mean (µg/mL)
Citrate Plasma	1:40,000	227
	1:80,000	237

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

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