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Cynomolgus Monkey Plasminogen Total Antigen ELISA Kit

Catalog No: CKK000A **Lot No:** TBD **Size:** 1 x 96-well kit **Expiration Date:** TBD

Sensitivity:	0.245 ng/ml, MDD
Range:	0.5-100 ng/ml
Sample Type:	Serum, plasma, and cell culture media
Cross Reactivity:	Not determined, studies in progress.

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:

Description	Quantity/kit
CKK000A – P. 96-well microtiter strip plate coated with anti- Cynomolgus Monkey plasminogen antibody, blocked and dried.	1 plate: 96 wells (12 strips x 8 wells)
CKK000A - A. Wash Buffer Concentrate (10x)	1 bottle, 50 mL
CKK000A - B. Cynomolgus Monkey plasminogen standard, lyophilized	1 vial
CKK000A - C. Biotinylated anti-Cyno Monkey plasminogen antibody, lyophilized.	1 vial
CKK000A - D. Horseradish peroxidase-conjugated Streptavidin, concentrated solution	1 vial
CKK000A - E. TMB substrate solution	1 bottle, 10 ml

Cell Sciences®
65 Parker Street
Unit 11
Newburyport, MA 02021

Toll Free: 888 769-1246
Phone: 978-572-1070
Fax: 978-992-0298

E-mail: info@cellsciences.com
Web Site: www.cellsciences.com

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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 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.

Reagents and Equipment Required:

- Pipettes covering 0-10 µl and 200-1000 µl, and tips
- 12-channel pipette covering 30-300µl
- Paper towels or laboratory wipes
- Polypropylene conical 50 ml tubes, 1.5 ml flip-cap tubes
- 1N H_2SO_4 or 1N HCl
- Bovine Serum Albumin Fraction V (BSA)
- Tris(hydroxymethyl)aminomethane (Tris)
- Sodium Chloride (NaCl)
- Deionized or distilled water
- Magnetic stirrer and stir-bars
- Plastic containers with lids
- Microtiter plate spectrophotometer operable at 450 nm
- Microtiter plate shaker with uniform horizontally circular movement up to 300 rpm
- Automatic plate washer or wash bottle

Warnings:

Warning – Avoid skin and eye contact when using TMB One 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.
- **DO NOT** smoke, drink, or eat in areas where specimens or reagents are being handled.

Preparation of Reagents:

- **TBS:** 0.1 M Tris 0.15 M NaCl, pH 7.4
- **Blocking buffer (BB):** 3% BSA (w/v) in TBS
- **Wash buffer 1X:** The wash buffer is supplied in a 10X concentrate. Dilute 50 ml of 10X wash buffer with 450 ml deionized water for use with the kit.

Specimen Collection:

Collect plasma using EDTA or citrate as an anticoagulant. Centrifuge for 15 minutes at 1,000 x g within 30 minutes of collection. Assay immediately or aliquot and store at ≤ - 20°C. Avoid repeated freeze-thaw cycles.

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Assay Procedure: Allow microtiter strips and assay components to warm to room temperature for 30 minutes. Perform assay at room temperature. Vigorously shake plate (300rpm) at each step of the assay.

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 1000 ng/ml standard solution.

Table 1: Dilution table for preparation of Cynomolgus Monkey Plasminogen Standard:

Plasminogen Concentration (ng/ml)	Dilutions
100	900 µl (BB) + 100 µl (from vial)
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)
0.5	500 µl (BB) + 500 µl (1 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.

Standard and Unknown Addition:
Remove microtiter plate from bag. Add 100 µl of 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 laboratory wipes.

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.

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Biotinylated Primary Antibody Addition:
Reconstitute antibody by adding **10 ml blocking buffer** to vial. 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 laboratory wipe.

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 **0.1 ml of 1:1,000** dilution to **9.9 ml of blocking buffer** to generate a 1:100,000 dilution. Add 100 µl of the 1:100,000 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 laboratory wipe.

Substrate Incubation:
Add 100 µl TMB substrate to all wells and shake plate for 2-10 minutes. Substrate will change from colorless to different intensities of blue. Quench reaction by adding 50 µl of 1N H_2SO_4 or 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 by gently shaking the plate and read plate immediately.

Measurement:
Set the absorbance at 450 nm on the microtiter plate reader. Measure the absorbance in all wells at 450 nm. Subtract zero point from all standards and unknowns to determine corrected absorbance (A_{450}).

Assay Calibration:
Plot A_{450} against the amount of Cynomolgus monkey plasminogen 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 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.

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.

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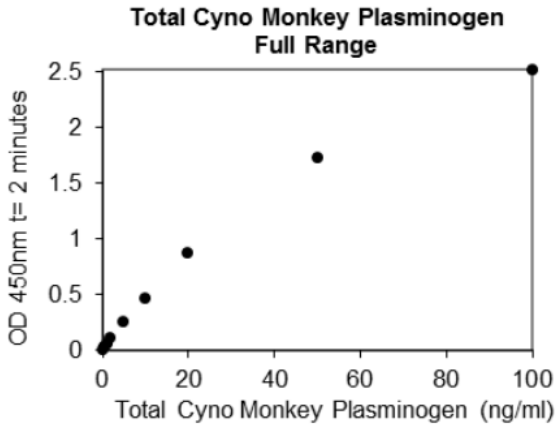
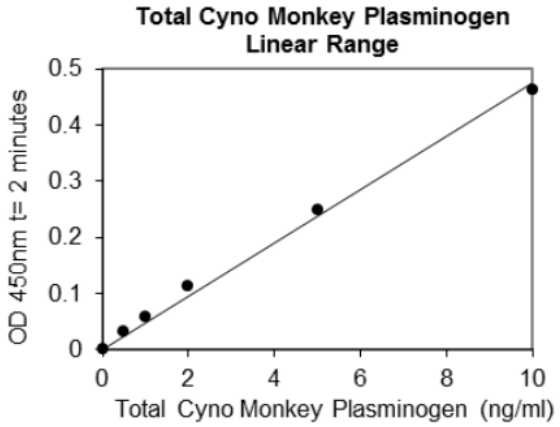
A typical standard curve.
(EXAMPLE ONLY, DO NOT USE)





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}: 0.137-0.1) and calculating the corresponding concentration. The MDD was 0.245 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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