

Cynomolgus Monkey IgG ELISA Kit

Catalog No: CKK001A **Lot No:** TBD **Size:** 1 x 96-well kit **Expiration Date:** 1 year
 CKK001B 5 x 96-well kit

Sensitivity:	0.095 ng/ml
Specificity:	Total cynomolgus macaque (<i>Macaca fascicularis</i>) IgG (Does not distinguish IgG subclasses.)
Range:	0.1-100 ng/ml
Sample Type:	Serum, plasma, hybridoma cell supernatants, ascites or other biological fluids
Cross-Reactivity:	Pooled normal plasma from Rhesus monkey was assayed and significant cross-reactivity was observed. Pooled normal plasma from human, mouse, rat, dog, sheep, pig and rabbit was assayed and no significant cross-reactivity was observed.

Background: IgG is the most abundant immunoglobulin in serum and is predominately involved in the secondary immune response. The IgG subclasses are designated 1, 2, 3 and 4 based on their relative prevalence in human serum.

Assay Principle: Cynomolgus monkey IgG will bind to the monoclonal capture antibody coated on the microtiter plate. After appropriate washing steps, biotin labeled monoclonal anti-Cynomolgus IgG primary antibody binds to the captured protein. Excess primary antibody is washed away and bound antibody is reacted with horseradish 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 monkey IgG. Color development is proportional to the concentration of IgG in the samples.

Reagents Provided:

Description	Quantity/kit
CKK001A - P. 96-well microtiter strip plate coated with anti- Cynomolgus Monkey IgG antibody, dried and blocked on well surface	1 plate: 96 wells (12 strips x 8 wells)
CKK001A - A. Wash Buffer Concentrate (10x)	1 bottle, 50 mL
CKK001A - B. Cynomolgus Monkey IgG standard, lyophilized	1 vial
CKK001A - C. Biotinylated anti-Cynomolgus Monkey IgG primary antibody, lyophilized.	1 vial
CKK001A - D. Horseradish peroxidase-conjugated Streptavidin, concentrated	1 vial
CKK001A - E. TMB substrate solution	1 bottle, 10 ml



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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 H₂SO₄ 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 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 **10 ml of blocking buffer** directly to the vial and agitate gently to completely dissolve contents. This will result in a 100 ng/ml standard solution.

Table 1: Dilution table for preparation of Cynomolgus Monkey IgG standard:

IgG Concentration (ng/ml)	Dilutions
100	100 μ l (from vial)
50	500 μ l (BB) + 500 μ l (from vial)
20	600 μ l (BB) + 400 μ l (50 ng/ml)
10	500 μ l (BB) + 500 μ l (20 ng/ml)
5	500 μ l (BB) + 500 μ l (10 ng/ml)
2	600 μ l (BB) + 400 μ l (5 ng/ml)
1	500 μ l (BB) + 500 μ l (2 ng/ml)
0.5	500 μ l (BB) + 500 μ l (1 ng/ml)
0.2	600 μ l (BB) + 400 μ l (0.5 ng/ml)
0.1	500 μ l (BB) + 500 μ l (0.2 ng/ml)
0	500 μ l (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 μ l of IgG 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 μ l wash buffer. Remove excess wash by gently tapping plate on paper towel or laboratory wipes.

NOTE: The assay measures total Cynomolgus IgG antigen in the 0.1 - 100 ng/ml range. If the unknown is thought to have higher IgG levels, dilutions may be made in blocking buffer. A 1:1,000,000 to 1:10,000,000 dilution for normal Cynomolgus monkey serum or plasma is suggested for best results.



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Biotinylated Anti-Cynomolgus Monkey Detection Monoclonal Antibody Addition:

Reconstitute antibody by adding **10 ml blocking buffer** to vial. Agitate gently to completely dissolve contents. Add 100 μ l to all wells. Shake plate at 300 rpm for 30 minutes. Wash wells three times with 300 μ l 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 μ l** of HRP-conjugated streptavidin into **2.5 ml blocking buffer** to generate a 1:1,000 dilution. Add **0.2 ml of 1:1,000** dilution to **9.6 ml of blocking buffer** to generate a 1:50,000 dilution. Add 100 μ l of the 1:50,000 dilution to all wells. Shake plate at 300 rpm for 30 minutes. Wash wells three times with 300 μ l wash buffer. Remove excess wash by gently tapping plate on paper towel or laboratory wipe.

Substrate Incubation:

Add 100 μ l 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 μ l of 1N H₂SO₄ 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 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 (A₄₅₀).

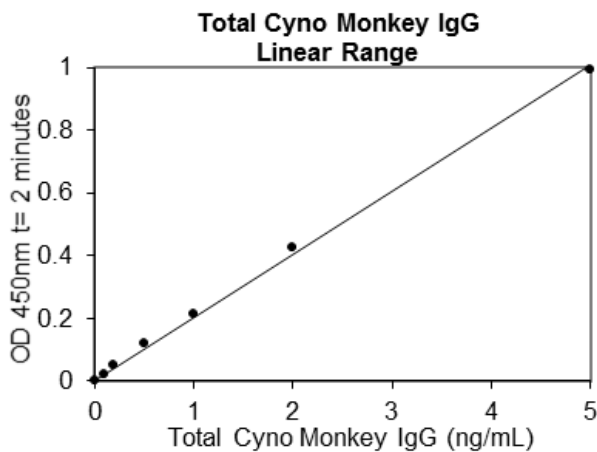
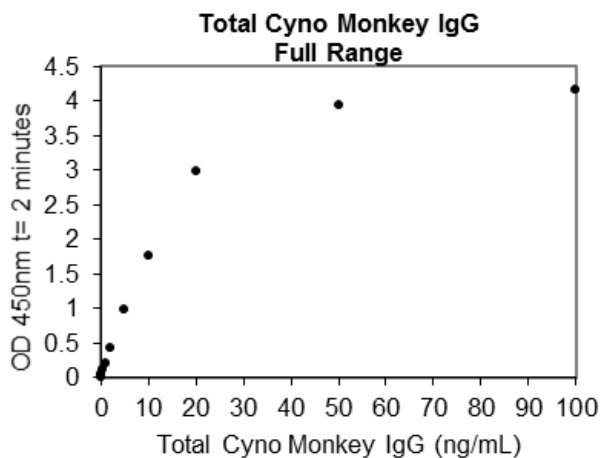
Assay Calibration:

Plot A₄₅₀ against the amount of Cynomolgus IgG 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 IgG in the unknowns can be determined from this curve. If samples have been diluted, the calculated concentration must be multiplied by the dilution factor.



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



**Expected
Values:**

The average concentration of IgG in normal Cynomolgus monkey serum is 15.6 mg/ml.



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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: 0.047-0.068) and calculating the corresponding concentration. The MDD was 0.095 ng/ml.

Specificity: This assay recognizes total Cynomolgus monkey IgG. Pooled normal plasma from human, mouse, rat, dog, sheep, pig and rabbit was assayed and no significant cross-reactivity was observed. Pooled normal plasma from Rhesus monkey was assayed and significant cross-reactivity was observed.

Intra-assay Precision: 3 samples of known concentration were tested 20 times on 1 plate to assess intra-assay precision.

Sample Type	1	2	3
n	20	20	20
Mean (ng/ml)	0.46	1.25	6.26
Standard Deviation	0.032	0.054	0.302
CV (%)	6.79	4.34	4.83

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

Sample Type	Dilution	Mean (mg/ml)
Citrate Plasma	1:2,500,000	11.4
	1:5,000,000	11.8

Disclaimer: This information is believed to be correct but does not claim to be all-inclusive and should be used only as a guide. The supplier of this kit shall not be held liable for any damage resulting from handling or from contact with the above product.

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