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Rhesus Monkey IgG ELISA Kit

Strip well format. Reagents for up to 96 tests

Catalog No.	**CKK002A**	**Quantity:**	**1 x 96 tests**
	CKK002B		**5 x 96 tests**

Intended Use: This rhesus macaque (*Macaca mulatta*) monkey Immunoglobulin G (IgG) antigen assay is intended for the quantitative determination of total IgG antigen in rhesus monkey serum, plasma, hybridoma cell supernatants, ascites or other biological fluids. The assay does not distinguish IgG subclasses.

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: Rhesus monkey IgG will bind to the monoclonal capture antibody coated on the microtiter plate. After appropriate washing steps, biotin labeled monoclonal anti-Rhesus 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 Rhesus monkey IgG. Color development is proportional to the concentration of IgG in the samples.

Reagents Provided:

- **96 well microtiter strip plate**
  8 x12 removable well strips containing anti-Rhesus Monkey IgG antibody coated on the blocked and dried surface.
- **10X Wash Buffer:**
  1 bottle of 50 ml; bring to 1X using DI water
- **Rhesus Monkey IgG antigen standard:**
  1 vial of lyophilized standard
- **Biotinylated anti-Rhesus Monkey IgG Detection monoclonal antibody:**
  1 vial of lyophilized biotinylated monoclonal antibody
- **Horseradish Peroxidase conjugated streptavidin:**
  1 vial of lyophilized HRP conjugated streptavidin
- **TMB substrate solution:**
  1 bottle of 10 ml solution

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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:

- 1-channel pipettes covering 0-10 µl and 200-1000 µl
- 12-channel pipette covering 30-300µl
- Paper towels or kimwipes
- 50 ml tubes, 1.5 ml centrifuge tubes
- 1N H_2SO_4 or 1N HCl
- DI 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.

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 concentrate: 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: 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 1,000 ng/ml standard solution.

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

IgG Concentration (ng/ml)	Dilutions
100	100 µl (from vial)
50	500 µl (BB) + 500 µl (from vial)
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)
0.2	600 µl (BB) + 400 µl (0.5 ng/ml)
0.1	500 µl (BB) + 500 µl (0.2 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.

Standard and Unknown Addition:
Remove microtiter plate from bag. Add 100 µl 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 µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.
NOTE: The assay measures total rhesus 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 rhesus monkey serum or plasma is suggested for best results.

Biotinylated Anti-Rhesus Monkey Detection Monoclonal 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 kimwipe.

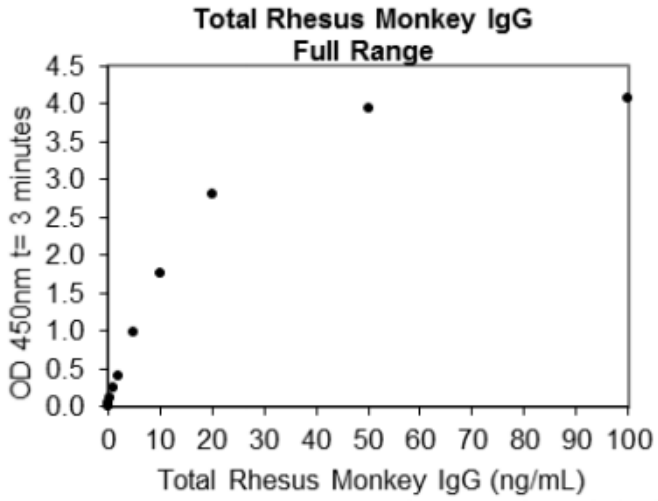
Streptavidin-HRP Addition:
Briefly centrifuge vial before opening. Dilute 2.5 µl of HRP conjugated streptavidin into 2.5 ml blocking buffer to generate a 1:1,000 dilution. Add 0.4 ml of 1:1,000 dilution to 9.6 ml of blocking buffer to generate a 1:25,000 dilution. Add 100 µl of the 1:25,000 dilution to all wells. 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.

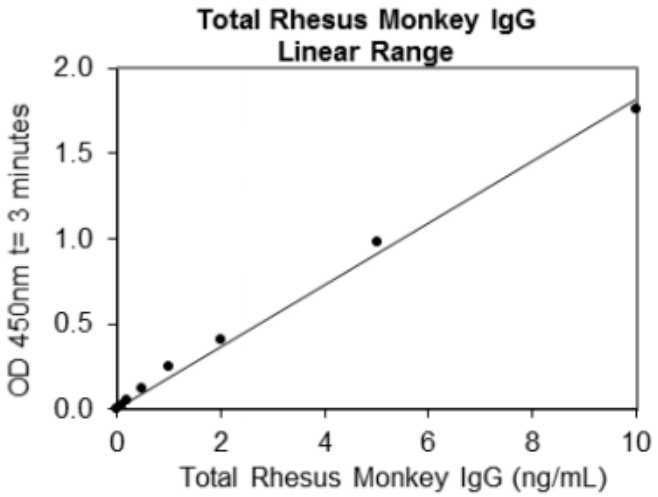
Substrate Incubation:
Add 100 µl TMB substrate to all wells and shake plate for 5-15 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50 µl of 1N H_2SO_4 or HCl stop solution to all wells when samples are visually in the same range as the standards. Add stop solution to wells in the same order as substrate upon which color will change from blue to yellow. Mix thoroughly 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}).

Assay Calibration:
Plot A_{450} against the amount of Rhesus 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.

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





Expected Values: The concentration of IgG in normal rhesus monkey serum ranges from 5 to 12 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: 0.046-0.059) and calculating the corresponding concentration. The MDD was 0.041 ng/ml.

Specificity: This assay recognizes total rhesus 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 cyno monkey was assayed and significant cross-reactivity was observed.

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	20.1
	1:5,000,000	23.1

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.

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

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