

Instruction Manual No. M-7060

Monkey IgM ELISA Kit

Cat. No. 7060, 96 tests

For Quantitative Determination of Monkey Immunoglobulin M in Serum, plasma or other biological fluids

For research use only (RUO), not for diagnosis, cure or prevention of the disease.



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www.4adi.com KIT CONTENTS

To Be Reconstituted: Store as indicated.

Component	Instructions for Use
Sample Diluent Concentrate (20x) Cat. No. SD-20T, 10ml	Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as Working Sample Diluent and store at 2-8°C until the kit lot expires or is used up.
Wash Solution Concentrate (100x) Cat. No. WB-100, 10ml	Dilute the entire volume 10ml + 990ml with distilled or deionized water into a clean stock bottle. Label as Working Wash Solution and store at RT until kit is used entirely.
Anti-Monkey IgM - HRP Conjugate Concentrate (100x) Part No. 7064, 0.15ml	Peroxidase conjugated anti-Monkey IgM in buffer with protein, detergents and BND as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of Working Sample Diluent is sufficient for 1 8-well strip. Use within the working day and discard. Return concentrate to 2-8°C storage.

Ready For Use: Store as indicated on labels.

Component	Part No.	Amt	Contents
Anti-Monkey IgM Microwell Strip Plate	7061	8-well strips (12)	Coated with purified anti-Monkey IgM antibodies.
Monkey IgM Standards			
50 ng/ml	7063B	0.65 ml	Five (5) vials, each containing purified monkey IgM with designated concentrations; diluted in buffer with protein, detergents and BND as stabilizers.
100 ng/ml	7063C	0.65 ml	
200 ng/ml	7063D	0.65 ml	
500 ng/ml	7063E	0.65 ml	
1000 ng/ml	7063F	0.65 ml	
Positive Control [IgM] range on label	7062	0.65 ml	Monkey IgM of stated concentration range; diluted in buffer with protein, detergents and BND as stabilizers.
TMB Solution	80091	12 ml	Chromogenic substrate for HRP containing TMB and peroxide.
Stop Solution	80101	12 ml	1% sulfuric acid.

STORAGE AND STABILITY

The microtiter well plate and all other reagents, if unopened, are stable at 2-8°C until the expiration date printed on the label. Stabilities of the working solutions are indicated under Reagent Preparation.

Materials Required But Not Provided:

- Pipettors and pipettes that deliver 100ul and 1-10ml. A multi-channel pipettor is recommended.
- Disposable glass or plastic 1-15ml tubes for diluting samples, anti-Monkey IgM-HRP Concentrate.
- Graduated cylinder to dilute Wash Concentrate and Sample Diluent Concentrate; 200ml to 1L.
- Stock bottle to store diluted Wash Solution; ; 200ml to 1L.
- Distilled or deionized water to dilute reagent concentrates.
- Microwell plate reader at 450 nm wavelength.

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature. Do not heat inactivate the serum. If sera are not assayed immediately, store refrigerated for up to 2 weeks, or frozen for long-term storage. Avoid freeze-thaw cycles. The use of plasma has not been investigated, but should be a suitable specimen for assay.

PRECAUTIONS AND SAFETY INSTRUCTIONS

Monkey serum may contain zoonotic, cross-species infectious material. Always wear gloves when handling serum-containing samples, including the standards and controls, and dispose of these samples and containers as biohazard waste.

Calibrators, Sample Diluent, and Antibody HRP contain bromonitrodioxane (BND: 0.05%, w/v). Stop Solution contains dilute sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water. MSDS for TMB, sulfuric acid and BND can be requested or obtained from the ADI website: Sample Diluent and anti-Protein G-HRP contain Proclin 300 (0.05%, v/v). <http://4adi.com/objects/catalog/product/extras/ELISA-Kit-SDS-MSDS-Set-1.pdf>

QUALITY CONTROL

Reagents Accurate and reproducible assay results rely on proper storage, handling and control of reagent and sample temperature. Store all reagents as indicated, and warm to room temperature only those to be used in the assay. Shelf-life of the critical reagents and samples will diminish with extended exposure to non-refrigeration, resulting in inaccurate assay results. All solutions should be clear. Cloudiness or particulates are indications of reagent contamination or instability and may interfere with proper performance of the assay. Do not use.

Sample Controls A Positive Serum Control is provided with the kit, assigned with an IgM concentration value range. Recovery in this range is an indicator of proper assay performance. Each lab should also assay internal control samples, which represent the lab's expected sample population and that are maintained stabilized. A Sample Diluent Negative Control should also be run.

Standard Curve The signal generated by the standards should be continuously increasing in OD from the lowest Standard to the highest Standard, with a difference greater than 1.2 OD. Non-continuously increasing or low signals may indicate problems with technique, protocol directions and/or reagent preparation, use or stability. A Negative Diluent Control should be of lower signal than the lowest standard. Do not rely on results generated from an assay with these issues.

Technique Accurate and reproducible assay results rely on good lab technique regarding pipetting, plate washing and handling of samples and reagents.

ASSAY PROCEDURE

Bring all reagents to lab temperature (18-30° C) equilibration (at least 30 minutes).

DILUTE Serum Samples in Working Sample Diluent. Dilutions of about 4k-fold are appropriate for most normal monkey sera. For accuracy, two dilution steps are recommended, as follows:

- 1) 10ul serum + 990ul diluent = [1:100],
- 2) 25ul [1:100] + 975ul diluent = [1:4k]

DO NOT dilute the Standards or Positive Control Serum.

ALL STEPS ARE PERFORMED AT ROOM TEMPERATURE. After each reagent addition, gently tap the plate to mix the well contents prior to beginning incubation.

1. Set-up

- Determine the number of wells for the assay run. Duplicates are recommended, including 10 Standard wells and 2 wells for each sample and control to be assayed.
- Remove the appropriate number of microwell strips from the pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated.
- Add 200-300ul Working Wash Solution to each well and let stand for about 5 minutes before sample addition.
- Aspirate the liquid and pat dry on a paper towel.

2. 1st Incubation [100ul – 60 min; 4 washes]

- Add 100ul of standards, samples and controls each to pre-determined wells.
- Tap the plate gently to mix reagents and incubate for 60 minutes.
- Wash wells 4 times and pat dry on fresh paper towels. As an alternative, an automatic plate washer may be used. Improper washes may lead to falsely elevated signals and poor reproducibility.

3. 2nd Incubation [100ul – 30 min; 5 washes]

- Add 100ul of diluted Anti-monkey IgM-HRP Conjugate to each well.
- Incubate for 30 minutes.
- Wash wells 5 times as in step 2.

4. Substrate Incubation [100ul – 15 min]

- Add 100ul TMB Substrate to each well. The liquid in the wells will begin to turn blue.
- Incubate for 15 minutes in the dark, e.g., place in a drawer or closet.

Note: If your microplate reader does not register optical density (OD) above 2.0, incubate for less time, or read OD at 405-410 nm (results are valid).

5. Stop Step [Stop: 100ul]

- Add 100ul of Stop Solution to each well.
- Tap gently to mix. The enzyme reaction will stop; liquid in the wells will turn yellow.

6. Absorbance Reading

- Use any commercially available microplate reader capable of reading at 450nm wavelength. Use a program suitable for obtaining OD readings, and data calculations if available.
- Read absorbance of the entire plate at 450nm using a single wavelength within 30 minutes after Stop Solution addition. If available, program to subtract OD at 630nm to normalize well background.

CALCULATION OF RESULTS

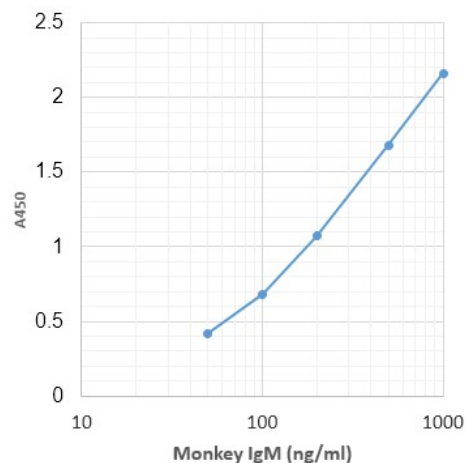
- The results may be calculated using any immunoassay software package. The four-parameter curve-fit is recommended. If software is not available, IgM concentrations may be determined as follows:
- Calculate the mean OD of duplicate samples.
- On graph paper plot the mean OD of the standards (y-axis) against the concentration (ng/ml) of IgM (x-axis). Draw the best fit curve through these points to construct the standard curve. A point-to-point construction is most common and reliable.
- The IgM concentrations in unknown samples and controls can be determined by interpolation from the standard curve.
- Multiply the values obtained for the samples by the dilution factor of each sample.
- Samples producing signals higher than the 1000 ng/ml standard should be further diluted and re-assayed.

TYPICAL RESULTS

The following data are for illustration purposes only. A complete standard curve should be run in every assay to determine sample values.

Wells	Standards, Control & Samples	A450 nm	IgM ng/ml
A1, A2	Negative Diluent Control	0.03	0
B1, B2	50 ng/ml Standard	0.42	50
C1, C2	100 ng/ml Standard	0.68	100
D1, D2	200 ng/ml Standard	1.07	200
E1, E2	500 ng/ml Standard	1.68	500
F1, F2	1000 ng/ml Standard	2.16	1000
G1, G2	Positive Serum Control [Value: 140 - 260 ng/ml]	1.13	218
H1, H2	Sample [Diluted 1:4k] Calculated: 4k-fold dilution x 349 ng/ml = 1.4 mg/ml in serum	1.44	349

A typical assay Standard Curve (do not use for calculating sample values)



PERFORMANCE CHARACTERISTICS

Specificity

The antibodies used in this kit have been shown by immunoelectrophoresis and ELISA to react specifically with IgM, and have essentially no reactivity with IgG, IgA, IgE or any other monkey serum proteins.

Serum from the following species showed no significant reactivity at 1:400 dilution: mouse, rat, hamster, guinea pig, bovine, pig, horse, sheep, goat, dog, cat, rabbit or chicken; also 10% neonatal bovine serum. Human serum shows extensive cross-reactivity.

Normal Range

Assay of IgM in stored sera from fourteen (14) individual Rhesus, Cynomolgous and Baboons ranged from 0.2 to 3.8 mg/ml (median = 1.85 mg/ml). Each laboratory should determine expected values of its own testing population.

Precision

Samples containing low, medium and high concentrations of IgM, representing 3 different species, were assayed multiple times in the same assay (n=10) to provide within-assay precision, and as duplicates in multiple assays (n=5) to obtain between-assay reproducibility. Coefficient of variations were calculated for the concentrations using a point-to-point curve-fitting program.

IgM concentrations were measured with good within-assay (5.3 to 7.8 %CV) and between-assay (4.2 to 16.2 %CV) reproducibility.

Sample	IgM ng/ml	Intra-assay %CV	Inter-assay %CV
Cynomolgous	199	7.1	6.9
Rhesus	811	5.3	4.2
Baboon	300	7.8	16.2

Linearity of Dilution

Four (4) individual stored sera were diluted to 2 levels for testing, and concordance of the assay values were compared. The mean recovery of the Monkey sera ranged from 94 to 100%, demonstrating linear dilution and equivalent quantification across the standard range. The Chimpanzee serum showed less agreement at 82% recovery.

Sample	Dilution	Assay Value ng/ml	Serum Value mg/ml	Concordance
Cynomolgous	1:4k	1062	4.25	95 %
	1:16k	292	4.68	
Rhesus	1:4k	698	2.79	100 %
	1:16k	175	2.80	
Baboon	1:4k	605	2.42	94 %
	1:16k	171	2.74	

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PERFORMANCE CHARACTERISTICS (continued)**Sample Recovery**

High and low concentrations of purified Monkey IgM were spiked into each of 3 serum samples. Observed assay values compared to expected values ranged from 83 to 105%, indicating accurate quantification of IgM in Monkey serum.

Sample	Expected ng/ml	Observed ng/ml	Observed/ Expected
High IgM Spike		433	
+ Rhesus M, 2042 ng/ml	2475	2064	83
+ Cynomol F, 2042 ng/ml	2475	2605	105
+ Baboon, 32.2 ng/ml	1893	1726	91
Low IgM Spike		77	
+ Rhesus M, 2042 ng/ml	2120	2098	99
+ Cynomol F, 2042 ng/ml	2120	2108	99
+ Baboon, 32.2 ng/ml	1537	1398	91

Related Items

Catalog#	Description
7010	Monkey IgA ELISA Kit, 96 tests, Quantitative
7050	Monkey IgG ELISA Kit, 96 tests, Quantitative
7050-RDT-25	TruStrip RDT Monkey IgG Rapid Test cards, 10/pk
7060	Monkey IgM ELISA Kit, 96 tests, Quantitative
7070	Monkey IgE ELISA Kit, 96 tests, Quantitative

For more details please consult our web site (www.4adi.com) or contact us by email (service@4adi.com).