Sample Recovery
High and low concentrations of Monkey CRP were mixed into each of 3 serum samples. Observed assay values compared to expected values ranged from 87 to 110%, indicating accurate quantification of CRP in monkey serum.

Sample	Expected ng/ml	Observed ng/ml	Observed/ Expected
High CRP Concn		18.5	
+ Rhesus serum 5.3 ng/ml	23.8	22.9	**96**
+ Cynomolgous serum 4.35 ng/ml	22.85	23.5	**103**
+ Baboon serum 6.25 ng/ml	24.75	27.2	**110**
Low CRP Concn		4.6	
+ Rhesus serum 5.3 ng/ml	9.9	8.9	**90**
+ Cynomolgous serum 4.35 ng/ml	8.95	7.75	**87**
+ Baboon serum 6.25 ng/ml	10.85	10.2	**94**

ELISA Kit Components	Amount	Cat/Part No.
Anti-Monkey CRP Microwell Strip Plate	8-well strips (12)	1051
Monkey CRP Standard, lyoph.	2 vials	1053
Anti-Monkey CRP HRP Conjugate (100X)	0.15 ml	1054
Sample Diluent Concentrate (20X)	10 ml	SD-20T
Wash Solution Concentrate (100X)	10 ml	WB-100
TMB Substrate	12 ml	80091
Stop Solution	12 ml	80101
Product Manual	1 ea	M-1050

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

Instruction Manual No. M-1050

Monkey CRP

ELISA Kit Cat. No. 1050

For Quantitative Determination of Monkey C-Reactive Protein (CRP) in Serum

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INTENDED USE

The Monkey CRP ELISA Kit is an in vitro immunoassay for research use in the quantification of monkey CRP, including rhesus, cynomolgous and baboon, circulating in serum or in other appropriately qualified samples from tissue fluids (e.g., saliva, mucosa), or in cultures of monkey cells.

RESEARCH USE OF THE TEST

C-reactive protein (CRP) is regarded as an acute phase reactant in serum (1), consisting as five non-covalently linked subunits, assembled as a cyclic pentamer, MW range of 110-140 kDa (2). CRP has been found to be increased in serum of patients with a wide variety of diseases including infections by gram-positive and gram-negative bacteria (3), acute phase of rheumatoid arthritis (4), abdominal abscesses, inflammation of bile ducts (4), myocardial infarction (4, 5), and malignant tumors (6, 7). CRP may be found in patients with Guillain-Barre syndrome and multiple sclerosis (8), certain viral infections (6, 9), tuberculosis (4, 7), acute infectious hepatitis (6), many other necrotic and inflammatory diseases, burned patients, and after surgical trauma (4). Although the detection of elevated levels of CRP in the serum is not specific for any particular disease, it is a useful indicator of inflammatory processes. CRP levels rise in serum within hours of the onset of inflammation, reach a peak during the acute stage and decrease with resolution of inflammation trauma. The detection of CRP is a more reliable and sensitive indicator of the inflammatory process than the erythrocyte sedimentation rate, which may also be influenced by physiological changes not associated with an inflammation process.

PRINCIPLE OF THE TEST

The Monkey CRP ELISA kit is based on the binding of Monkey CRP in samples to two antibodies, one immobilized on the microtiter wells, and the other conjugated to horseradish peroxidase (HRP) enzyme. After a washing step, chromogenic substrate is added and color is developed by the enzymatic reaction of HRP on the TMB substrate, which is directly proportional to the amount of CRP present in the sample. Stopping Solution is added to terminate the reaction, and absorbance at 450nm is then measured using an ELISA microtiter well reader. The concentration of CRP in samples and control is calculated from a curve of standards containing known concentrations of CRP.

STORAGE AND STABILITY

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

PERFORMANCE CHARACTERISTICS & EXPECTED RESULTS

Specificity
The antibodies used in this kit have been shown by immunoelectrophoresis and ELISA to react specifically with CRP, 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, pig, horse, sheep, goat, dog, cat or rabbit; also 10% neonatal bovine serum. Human and chimpanzee sera showed substantial reactivity.

Normal Range
Assay of CRP in stored, frozen sera from seventeen (17) individual adult rhesus, cynomolgous and baboons ranged from 3.8 to 46.9 ug/ml (median = 14.5 ug/ml). Each laboratory should determine expected values of its own testing population.

Precision
Samples containing low, medium and high concentrations of CRP 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. Coefficients of variation (CVs) were calculated for the concentrations using a point-to-point curve-fitting program.

CRP concentrations were measured with good within-assay (6.6 to 7.8 %CV) and between-assay (4.6 to 8.0 %CV) reproducibility.

Sample	CRP ng/ml	Intra-assay %CV	Inter-assay %CV
Low Sample	7.0	**7.4**	**8.0**
Mid Sample	13.1	**6.6**	**4.6**
High Sample	29.2	**7.8**	**5.3**

Linearity of Dilution
Three (3) individual stored sera were diluted to 2 levels for testing, and concordance of the assay values was compared. Agreement of values ranged from 94 to 98%, demonstrating linear dilution and equivalent quantification across the standard range.

Sample	Dilution	Assay Value ng/ml	Serum Value ug/ml	Concordance
Rhesus	1:375 1:3k	85 11	31.7 32.7	**98 %**
Cynomolgous	1:1k 1:8k	36 5.1	36.4 40.8	**94 %**
Baboon	1:750 1:6k	61 6.9	45.8 41.4	**95 %**

CALCULATIONS

The results may be calculated using any immunoassay software package. The four-parameter curve-fit is recommended. If software is not available, Monkey CRP concentrations may be determined as follows:

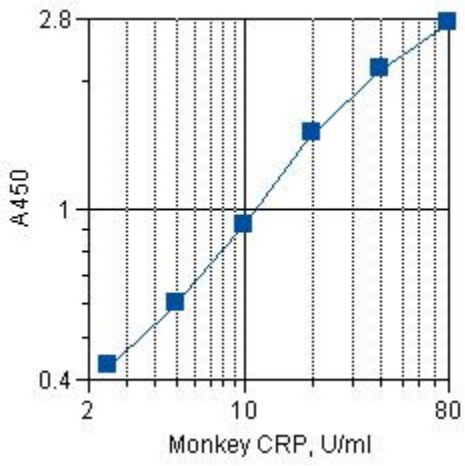
1. Calculate the mean OD of duplicate samples.
2. On graph paper plot the mean OD of the standards (y-axis) against the concentration (ng/ml) of Monkey CRP (x-axis). Draw the best fit curve through these points to construct the standard curve. A point-to-point construction is most common and reliable.
3. The Monkey CRP concentrations in unknown samples and controls can be determined by interpolation from the standard curve.
4. Multiply the values obtained for the samples by the dilution factor of each sample.
5. Samples producing signals higher than the 80 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	CRP ng/ml
1,2 A	**Negative Diluent Control**	0.29	0
1,2 B	2.5 ng/ml **Standard**	0.43	2.5
1,2 C	5 ng/ml **Standard**	0.60	5
1,2 D	10 ng/ml **Standard**	0.92	10
1,2 E	20 ng/ml **Standard**	1.51	20
1,2 F	40 ng/ml **Standard**	2.13	40
1,2 G	80 ng/ml **Standard**	2.73	80
1,2 H	**Sample** [Diluted 1:1k]	1.20	14
	Calculated: 1k-fold dilution x 14 ng/ml = **14** ug/ml in serum		

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



KIT CONTENTS

To Be Reconstituted: Store as indicated.

Component	Instructions for Use
Monkey CRP Standard Part No. 1053	Two (2) vials, each containing monkey CRP lyophilized in buffer with protein as stabilizers. Keep lyophilized vials refrigerated until used or kit lot expires.

Reconstitute 1 vial with 1.0 ml **Working Sample Diluent** to provide an 80 ng/ml Top Standard, sufficient for two curves in duplicate. Prepare 2-fold dilutions, as follows:

Standard	+ Diluent	= Final Concn
Reconstituted Standard	None	80 ng/ml
225 ul of 80 ng/ml	225ul	40 ng/ml
225 ul of 40 ng/ml	225ul	20 ng/ml
225 ul of 20 ng/ml	225ul	10 ng/ml
225 ul of 10 ng/ml	225ul	5 ng/ml
225 ul of 5 ng/ml	225ul	2.5 ng/ml

Use within 30 days of preparation; store @ 4C.

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, to 1L with distilled or deionized water into a clean stock bottle. Label as **Working Wash Solution** and store at ambient temperature until kit is used entirely.
Anti-Monkey CRP-HRP Conjugate Concentrate (100x) Part No. 1054, 0.15ml	Peroxidase conjugated antibody reactive with monkey CRP in buffer with protein, detergents and antimicrobial 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 #	Amt	Contents
Anti-Monkey CRP Microwell Strip Plate	1051	8-well strips (12)	Coated with purified antibodies reactive with monkey CRP. Return unused strips to the pouch with desiccant; re-seal and store refrigerated.
TMB Substrate	80091	12 ml	Chromogenic substrate for HRP containing TMB and peroxide.
Stop Solution	80101	12 ml	1% sulfuric acid.

Materials Required But Not Provided:

- Pipettors and pipettes that deliver 100ul and 1-10ml. A multi-channel pipettor is recommended.
- Disposable glass or plastic 5-15ml tubes for diluting samples and Antibody-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.

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.

Standards, Controls, Sample Diluent, and Antibody-HRP contain Bromo-nitro-dioxane (BND: 0.05%, w/v). Stop Solution contains 1% 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, if not already on file, can be requested or obtained from the ADI website.

SPECIMEN COLLECTION AND HANDLING

Culture medium, serum and other biological fluids may be used as samples with proper dilution to avoid solution matrix interference.

For **serum**, collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature.

For **other samples**, including tissue culture media, clarify the sample by centrifugation and/or filtration prior to dilution in Working Sample Diluent. If samples will not be assayed immediately , store refrigerated for up to a week, or frozen for long-term storage. Avoid freeze-thaw cycles.

ASSAY PROCEDURE

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

DILUTE Serum Samples in Working Sample Diluent. Dilutions of about 1:1k are appropriate for most normal monkey sera. For accuracy, two dilution steps are recommended, as follows:

1) 10ul serum + 490ul diluent = [1:50],
2) 25ul [1:50] + 475ul diluent = **[1:1k].**

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, to include 12 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 about 5 to 15 minutes before sample addition.
- Aspirate or dump the liquid and pat the plate 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 – 60 min; 5 washes]

- Add 100ul of Working Anti-Monkey CRP-HRP Conjugate to each well.
- Incubate for 60 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.