LSBio™ Mouse CRP / C-Reactive Protein ELISA Kit

Catalog No. LS-F10439

User Manual

Please Read the Manual Carefully
Before Starting your Experiment

LSBio
LifeSpan BioSciences, Inc.

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Mouse C-Reactive Protein Antigen ELISA Kit

Catalog # LS-F10439 Strip well format. Reagents for up to 96 tests.

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INTENDED USE

This mouse C-reactive protein (CRP) antigen assay is intended for the quantitative determination of total CRP in mouse plasma and other biological fluids. **For research use only.**

BACKGROUND

CRP is an acute phase reactant which is elevated in plasma in response to increased interleukin-6 induced by inflammation, infection and tissue injury [1]. CRP is expressed mainly in the liver and activates the complement pathway following calcium-dependent binding to phosphocholine on apoptotic, necrotic and microbial cells.

ASSAY PRINCIPLE

Mouse CRP will bind to the affinity purified capture antibody coated on the microtiter plate. After appropriate washing steps, biotin labeled polyclonal anti-mouse CRP primary antibody binds to the captured protein. Excess primary antibody is washed away and bound antibody is reacted with peroxidase conjugated streptavidin. Following an additional washing step, TMB substrate is used for color development at 450nm. A standard calibration curve is prepared along with the samples to be measured using dilutions of mouse CRP. Color development is proportional to the concentration of CRP in the samples.

REAGENTS PROVIDED

- **96-Well antibody coated microtiter strip plate** (removable wells 8x12) containing anti-mouse CRP antibody, blocked and dried.
- **10X Wash buffer:** 1 bottle of 50ml
- **Mouse CRP standard:** 1 vial lyophilized standard
- **Anti-mouse CRP primary antibody:** 1 vial lyophilized polyclonal antibody
- **Horseradish peroxidase-conjugated streptavidin:** 1 vial concentrated HRP labeled streptavidin
- **TMB substrate solution:** 1 bottle of 10ml solution

STORAGE AND STABILITY

Store all kit components at 4°C upon arrival. Return any unused microplate strips to the plate pouch with desiccant. Reconstituted standard and primary may be stored at -80°C for later use. Do not freeze-thaw the standard and primary more than once. Store all other unused kit components at 4°C. This kit should not be used beyond the expiration date.

OTHER REAGENTS AND SUPPLIES REQUIRED

- Microtiter plate shaker capable of 300 rpm uniform horizontally circular movement
- Manifold dispenser/aspirator or automated microplate washer
- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes and Pipette tips
- Deionized or distilled water
- Polypropylene tubes for dilution of standard
- Paper towels or laboratory wipes
- 1N H_2SO_4 or 1N HCl

PRECAUTIONS

- FOR LABORATORY RESEARCH USE ONLY. NOT FOR DIAGNOSTIC USE.
- 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.
- Always pour peroxidase substrate out of the bottle into a clean test tube. Do not pipette out of the bottle as contamination could result.
- Keep plate covered except when adding reagents, washing, or reading.
- DO NOT pipette reagents by mouth and avoid contact of reagents and specimens with skin.
- DO NOT smoke, drink, or eat in areas where specimens or reagents are being handled.

PREPARATION OF REAGENTS

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

SAMPLE COLLECTION

Collect plasma using EDTA, citrate, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. Assay immediately or aliquot and store at ≤ -20°C. Avoid repeated freeze-thaw cycles.

ASSAY PROCEDURE

Perform assay at room temperature. Vigorously shake plate (300rpm) at each step of the assay.

Preparation of Standard

Reconstitute standard by adding 1.25ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. This will result in a 4ng/ ml standard solution.

Dilution table for preparation of mouse CRP standard:

CRP concentration (ng/ml)	Dilutions
4	100 µl from standard vial
2	500µl (BB) + 500µl (4ng/ml)
1	500µl (BB) + 500µl (2ng/ml)
0.5	500µl (BB) + 500µl (1ng/ml)
0.2	600µl (BB) + 400µl (0.5ng/ml)
0.1	500µl (BB) + 500µl (0.2ng/ml)
0.05	500µl (BB) + 500µl (0.1ng/ml)
0.02	600µl (BB) + 400µl (0.05ng/ml)
0	500µl (BB) Zero point to determine background

NOTE: DILUTIONS FOR THE STANDARD CURVE AND ZERO STANDARD MUST BE MADE AND APPLIED TO THE PLATE IMMEDIATELY.

Standard and Unknown Addition

Remove microtiter plate from bag and add 100µl CRP standards (in duplicate) and unknowns to wells. Carefully record position of standards and unknowns. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

NOTE: The assay measures CRP antigen in the 0.02-4 ng/ml range. Samples giving mouse CRP levels above 4 ng/ml should be diluted in blocking buffer before use. A 1:20,000 to 1:80,000 dilution for normal mouse plasma is suggested for best results.

Primary Antibody Addition

Reconstitute primary antibody by adding 10ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. Add 100µl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Streptavidin-HRP Addition

Briefly centrifuge vial before opening. Dilute 2.5µl of HRP conjugated streptavidin into 2.5ml blocking buffer to generate a 1:1,000 dilution. Add 0.4ml of 1:1,000 dilution to 9.6ml of blocking buffer to generate a 1:25,000 dilution. Add 100µl of the 1:25,000 dilution to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Substrate Incubation

Add 100µl TMB substrate to all wells and shake plate for 4-10 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50µl 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.

Measurement

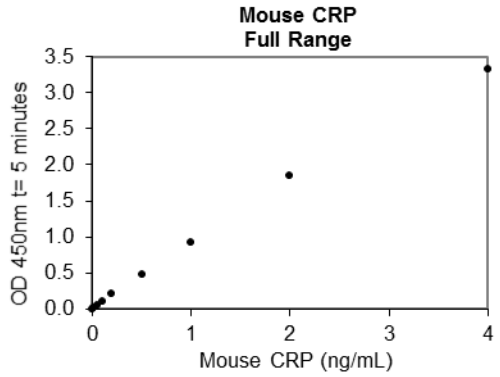
Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm. Subtract zero point from all standards and unknowns to determine corrected absorbance (A_{450}).

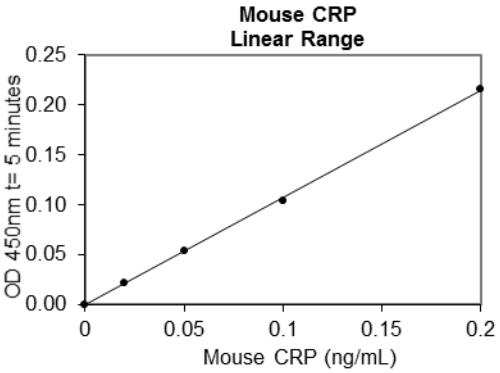
Calculation of Results

Plot A_{450} against the amount of mouse CRP 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 CRP 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):





EXPECTED VALUES

The concentration of CRP in normal human plasma was found to be 1-2 µg/ml, is higher in females and increases with age [2]. CRP is elevated to 10-40 µg/ml by mild inflammation and viral infections, 40-200 µg/ml by active inflammation and bacterial infection, and >200 µg/ml by

severe bacterial infections and burns [3].Normal values of CRP in mouse plasma have not been conclusively determined.

PERFORMANCE CHARACTERISTICS

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}: 0.05-0.061) and calculating the corresponding concentration. The MDD was 0.001ng/ml.

Precision: These studies are currently in progress. Please contact us for more information.

Inter-assay Precision: These studies are currently in progress. Please contact us for more information.

Recovery: These studies are currently in progress. Please contact us for more information.

Linearity: To assess the linearity of the assay, mouse plasma samples containing high concentrations of antigen were serially diluted to produce samples with values within the dynamic range of the assay.

Sample	1:2	1:4	1:8	1:16
n	4	4	4	4
Average % of expected	102	84	94	95
Range	97-107%	75-90%	91-96%	82-106%

Specificity: These studies are currently in progress. Please contact us for more information.

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

Sample Type	Dilution	Mean(µg/ml)
C57 Mouse Citrate Plasma	1:25,000	20.5
	1:50,000	17.2
Balb/C Mouse Citrate Plasma	1:25,000	3.5
	1:50,000	3.4
CD1 Mouse Citrate Plasma	1:25,000	7.2
	1:50,000	6.2

DISCLAIMER

This information is believed to be correct but does not claim to be all-inclusive and shall be used only as a guide.

The supplier of this kit shall not be held liable for any damage resulting from handling of or contact with the above product.

REFERENCES

1. Thompson D, *et al*.: Structure 1999, 7(2):169-177. 2. Hutchinson WL, *et al*.: Clinical Chemistry 2000, 46(7): 934-938.

3. Clyne B & Olshaker JS: J. Emerg. Med. 1999, **Intra-assay** 17(6):1019-1025.

Example of ELISA Plate Layout
96 Well Plate: 18 Standard wells, 78 Sample wells

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0.02 ng/ml	0.05 ng/ml	0.1 ng/ml	0.2 ng/ml	0.5 ng/ml	1 ng/ml	2 ng/ml	4 ng/ml			
B	0	0.02 ng/ml	0.05 ng/ml	0.1 ng/ml	0.2 ng/ml	0.5 ng/ml	1 ng/ml	2 ng/ml	4 ng/ml			
C												
D												
E F												
G												
H												

Important Note: During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. We recommend briefly centrifuging the vial to dislodge any liquid in the container's cap prior to opening.

Warning: This reagent may contain sodium azide and sulfuric acid. The chemical, physical, and toxicological properties of these materials have not been thoroughly investigated. Standard Laboratory Practices should be followed. Avoid skin and eye contact, inhalation, and ingestion.

Sodium azide forms hydrazoic acid under acidic conditions and may react with lead or copper plumbing to form highly explosive metal azides. On disposal, flush with large volumes of water to prevent accumulation.

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