Quantikine® ELISA

Mouse C1q R1/CD93 Immunoassay

Catalog Number MCD930

For the quantitative determination of mouse Complement component C1 q Receptor 1 (C1q R1) concentrations in cell culture supernates, tissue lysates, serum, and plasma.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

TABLE OF CONTENTS

SECTION	PAGE
INTRODUCTION	1
PRINCIPLE OF THE ASSAY	2
LIMITATIONS OF THE PROCEDURE	2
TECHNICAL HINTS	2
PRECAUTION	2
MATERIALS PROVIDED & STORAGE CONDITIONS	3
OTHER SUPPLIES REQUIRED	3
SAMPLE COLLECTION & STORAGE	4
SAMPLE PREPARATION	4
REAGENT PREPARATION	5
ASSAY PROCEDURE	6
CALCULATION OF RESULTS	7
TYPICAL DATA	7
PRECISION	8
RECOVERY	8
LINEARITY	8
SENSITIVITY	9
CALIBRATION	9
SAMPLE VALUES	9
SPECIFICITY	10
REFERENCES	10

MANUFACTURED AND DISTRIBUTED BY:

USA & Canada | R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413, USA
TEL: (800) 343-7475 (612) 379-2956 FAX: (612) 656-4400
E-MAIL: info@RnDSystems.com

DISTRIBUTED BY:

UK & Europe | R&D Systems Europe, Ltd.
19 Barton Lane, Abingdon Science Park, Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449 FAX: +44 (0)1235 533420
E-MAIL: info@RnDSystems.co.uk

China | R&D Systems China Co., Ltd.
24A1 Hua Min Empire Plaza, 726 West Yan An Road, Shanghai PRC 200050
TEL: +86 (21) 52380373 FAX: +86 (21) 52371001
E-MAIL: info@RnDSystemsChina.com.cn

INTRODUCTION

C1q R1, also known as CD93, C1qRp, and AA4, is an approximately 125 kDa transmembrane glycoprotein that plays several roles in immune system function (1). Mature mouse CD93 consists of a 550 amino acid (aa) extracellular domain (ECD) with one C-type lectin domain, five tandem EGF-like domains, and a mucin-like domain, followed by a 21 aa transmembrane segment and a 51 aa cytoplasmic domain (2, 3). Within the ECD, mouse CD93 shares 66% and 87% aa sequence identity with human and rat CD93, respectively. It is distinct from the 60 kDa Calreticulin which is likewise known as C1q R1 (4, 5). Unlike Calreticulin, and despite its name, C1q R1/CD93 does not appear to bind the complement protein C1q (5, 6). CD93 is expressed by vascular endothelial cells (5, 7, 8) and by a variety of hematopoietic cells including monocytes, granulocytes, immature dendritic cells, NK cells, platelets, hematopoietic progenitor cells, naïve T cells, immature B cells, and plasma cells (4-6, 9-11). It is weakly expressed on resident tissue and peritoneal macrophages but is upregulated following inflammatory activation (5, 13).

Various sized fragments of soluble CD93 (50-75 kDa) can be shed from monocytes, neutrophils, and vascular endothelial cells following inflammatory stimulation, leaving a residual stub in the membrane (14-16). Soluble CD93 levels are elevated in peritoneal lavage fluid during peritonitis, in serum during sepsis and systemic sclerosis, and in synovial fluid during rheumatoid arthritis relative to osteoarthritis (15-18). Moderate serum elevation of soluble CD93 is correlated with a decreased risk of coronary artery disease and myocardial infarction (19).

Cross-linking of cell surface CD93 enhances phagocytosis by monocytes and enhances the uptake of apoptotic cells *in vivo* (13, 20, 21). Soluble CD93 binds directly to monocyte and vascular endothelial cell surfaces (5, 15). It promotes the differentiation of monocytes to macrophages, phagocytosis of apoptotic cells, and inflammatory responsiveness to multiple TLR ligands (15, 18). Although it contributes to these aspects of inflammation, CD93 is not required for macrophage recruitment during thioglycollate-induced peritonitis (13, 20). It is also not required for plasma cell differentiation but is required for their longevity in the bone marrow and for the resulting extended humoral response (12).

The Quantikine Mouse C1q R1/CD93 Immunoassay is a 3.5 hour solid phase ELISA designed to measure mouse C1q R1 levels in cell culture supernates, tissue lysates, serum, and plasma. It contains NS0-expressed recombinant mouse C1q R1 and antibodies raised against the recombinant factor. This immunoassay has been shown to quantitate the recombinant mouse C1q R1 accurately. Results obtained using natural mouse C1q R1 showed dose-response curves that were parallel to the standard curves obtained using the recombinant kit standards. These results indicate that this kit can be used to determine relative mass values for natural mouse C1q R1.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse C1q R1 has been pre-coated onto a microplate. Standards, Control, and samples are pipetted into the wells and any mouse C1q R1 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse C1q R1 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the Stop Solution is added. The intensity of the color measured is in proportion to the amount of mouse C1q R1 bound in the initial step. The sample values are then read off the standard curve.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, further dilute the samples with Calibrator Diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution.

PRECAUTION

The Stop Solution provided with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/RECONSTITUTED MATERIAL
Mouse C1q R1 Microplate	894375	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse C1q R1.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Mouse C1q R1 Conjugate	894376	12 mL of a polyclonal antibody specific for mouse C1q R1 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Mouse C1q R1 Standard	894377	25 ng of recombinant mouse C1q R1 in a buffered protein base with preservatives; lyophilized.	
Mouse C1q R1 Control	894378	Recombinant mouse C1q R1 in a buffered protein base with preservatives; lyophilized. The concentration range of mouse C1q R1 after reconstitution is shown on the vial label. The assay value of the Control should be within the range specified on the label.	
Assay Diluent RD1W	895038	12 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-26 Concentrate	895525	21 mL of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895024	50 mL of a 25-fold concentrated solution of buffered surfactant with preservative.	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895174	23 mL of diluted hydrochloric acid.	
Plate Sealers	N/A	4 adhesive strips.	

* Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 1000 mL graduated cylinders.
- Test tubes for dilution of standards and samples.

If using tissue lysate samples, the following are also required:

- Cell Lysis Buffer 2 (R&D Systems, Catalog # 895347).
- PBS

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Tissue Lysates - Prior to assay, tissues must be lysed according to the directions in the Sample Values section.

Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 2000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Strict adherence to clotting time should be observed as C1q R1 levels increase with extended clotting times.*

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 20 minutes at 2000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.*

SAMPLE PREPARATION

Serum and plasma samples require a 50-fold dilution. A suggested 50-fold dilution is 10 µL of sample + 490 µL of Calibrator Diluent RD5-26 (1X).

Tissue lysate samples require at least a 2-fold dilution. A suggested 2-fold dilution is 75 µL of sample + 75 µL of Calibrator Diluent RD5-26 (1X).

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Mouse C1q R1Kit Control - Reconstitute the Kit Control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the Control undiluted.

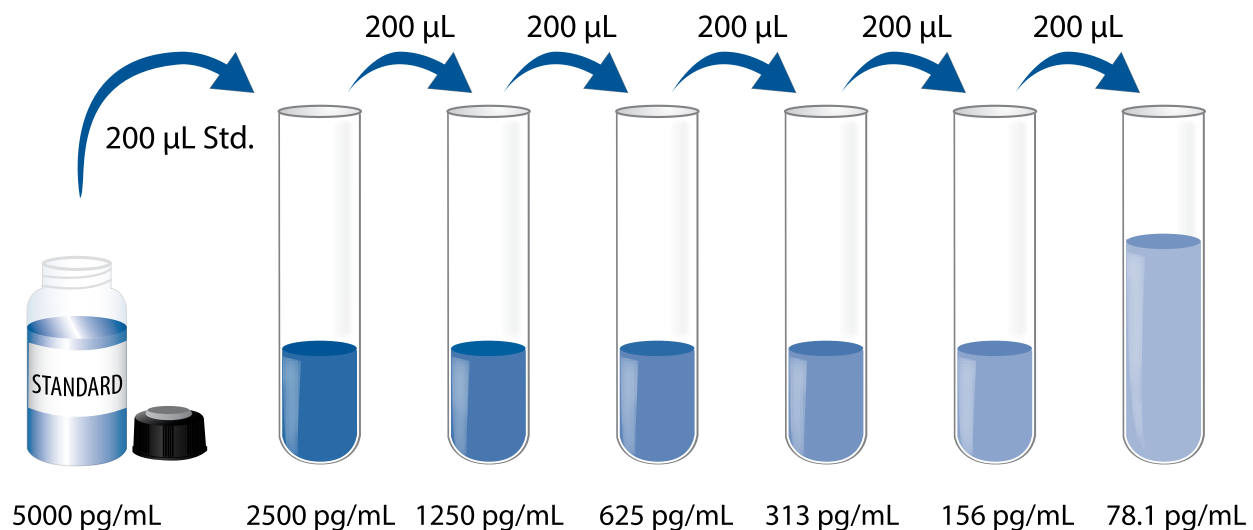
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. To prepare enough Wash Buffer for one plate, add 25 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 625 mL of Wash Buffer.

Calibrator Diluent RD5-26 (1X) - Dilute 20 mL of Calibrator Diluent RD5-26 Concentrate into 60 mL of deionized or distilled water to prepare 80 mL of Calibrator Diluent RD5-26 (1X).

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 µL of the resultant mixture is required per well.

Mouse C1q R1 Standard - Reconstitute the Mouse C1q R1 Standard with 5.0 mL of Calibrator Diluent RD5-26 (1X). This reconstitution produces a stock solution of 5000 pg/mL. Allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Pipette 200 µL of Calibrator Diluent RD5-26 (1X) into each tube. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. The 5000 pg/mL standard serves as the high standard. Calibrator Diluent RD5-26 (1X) serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, Control, and samples be assayed in duplicate.

1. Prepare all reagents, working standards, Control, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 50 µL of Assay Diluent RD1W to each well.
4. Add 50 µL of Standard, Control, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature.
5. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of Mouse C1q R1 Conjugate to each well. Cover with a new adhesive strip. Incubate for 1 hour at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature on the benchtop. **Protect from light.**
9. Add 100 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Samples may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

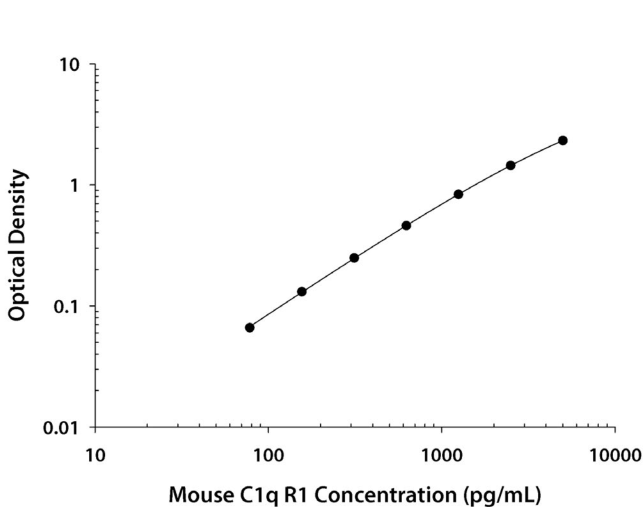
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density.

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the mouse C1q R1 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted prior to assay, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D.	Average	Corrected
0	0.010 0.010	0.010	—
78.1	0.076 0.076	0.076	0.066
156	0.140 0.141	0.141	0.131
313	0.257 0.260	0.259	0.249
625	0.470 0.470	0.470	0.460
1250	0.843 0.846	0.845	0.835
2500	1.447 1.462	1.455	1.445
5000	2.308 2.352	2.330	2.320

PRECISION

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in twenty-two separate assays to assess inter-assay precision.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	22	22	22
Mean (pg/mL)	248	704	2033	256	692	2085
Standard deviation	2.81	13.2	29.2	19.2	33.2	116
CV (%)	1.1	1.9	1.4	7.5	4.8	5.6

RECOVERY

The recovery of mouse C1q R1 spiked to three levels throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Cell culture samples (n=8)	95	84-105%
Tissue lysates (n=4)	110	102-116%

LINEARITY

To assess the linearity of the assay, samples containing high concentrations of mouse C1q R1 were serially diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay. Samples were diluted prior to assay.

		Cell culture supernates (n=4)	Tissue lysates (n=4)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)
1:2	Average % of Expected	105	107	102	98	98
	Range (%)	102-108	104-111	98-105	96-101	94-101
1:4	Average % of Expected	105	108	100	98	97
	Range (%)	103-108	105-115	96-104	95-102	93-101
1:8	Average % of Expected	109	108	100	97	96
	Range (%)	103-112	106-113	96-101	92-104	88-102
1:16	Average % of Expected	110	104	99	97	98
	Range (%)	107-112	101-108	96-101	93-102	91-102

For research use only. Not for use in diagnostic procedures.

SENSITIVITY

Sixty assays were evaluated and the minimum detectable dose (MDD) of mouse C1q R1 ranged from 0.856-11.4 pg/mL. The mean MDD was 2.94pg/mL.

The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified NS0-derived recombinant mouse C1q R1 produced at R&D Systems.

SAMPLE VALUES

Serum/Plasma - Samples were evaluated for the presence of C1q R1 in this assay.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=10)	143	92.8-238	45.2
EDTA plasma (n=10)	103	62.0-152	26.7
Heparin plasma (n=10)	90.5	47.7-147	40.9

Cell Culture Supernates:
Organs from mice were removed, rinsed in PBS, and kept on ice. The organs were then homogenized using a tissue homogenizer and cultured in RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin sulfate. Cells were cultured for 3 days. Aliquots of the cell culture supernates were removed and assayed for levels of natural C1q R1.

Tissue Type	(pg/mL)
Lung	17,606
Spleen	1416

P388D1 mouse lymphoma cells (1 x 10^5 cells/flask) were cultured in RPMI supplemented with 10% equine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin sulfate, and incubated for 7 days. Cells were cultured for 3 days. An aliquot of the cell culture supernate was removed, assayed for levels of natural C1q R1, and measured 1200 pg/mL.

Tissue Lysates - Organs from mice were rinsed with PBS and homogenized with a tissue homogenizer in PBS. An equal volume of Cell Lysis Buffer 2 was added and tissues were lysed at room temperature for 30 minutes with gentle agitation. Debris was then removed by centrifugation. Aliquots of the lysates were removed and assayed for levels of natural C1q R1.

Tissue Type	(ng/mL)
Lung	135
Spleen	5.24

SPECIFICITY

This assay recognizes natural and recombinant mouse C1q R1.

The factors listed below were prepared at 500 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 500 ng/mL in a mid-range recombinant mouse C1q R1 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:

C1qL2
C1qTNF9
Complement Component C1ra
Complement Component C1r LP
Complement Component C5a
CORS26/C1qTNF3
MBL-1
MBL-2

Recombinant human:

C1q R1/CD93
Complement Component C1qA
Complement Component C1qC
Complement Component C3a

REFERENCES

1. Greenlee-Wacker, M.C. *et al.* (2012) Curr. Drug Targets **13**:411.
2. Petrenko, O. *et al.* (1999) Immunity **10**:691.
3. Norsworthy, P.J. *et al.* (1999) Mamm. Genome **10**:789.
4. Nepomuceno, R.R. and A.J. Tenner (1998) J. Immunol. **160**:1929.
5. McGreal, E.P. *et al.* (2002) J. Immunol. **168**:5222.
6. Steinberger, P. *et al.* (2002) J. Leukoc. Biol. **71**:133.
7. Xiao, S. *et al.* (2001) Clin. Exp. Immunol. **125**:360.
8. Harhausen, D. *et al.* (2010) J. Immunol. **184**:6407.
9. Lovik, G. *et al.* (2001) Scand. J. Immunol. **53**:410.
10. Danet, G.H. *et al.* (2002) Proc. Natl. Acad. Sci. USA **99**:10441.
11. Ikewaki, N. *et al.* (2010) J. Clin. Immunol. **30**:723.
12. Chevrier, S. *et al.* (2009) Proc. Natl. Acad. Sci. USA **106**:3895.
13. Norsworthy, P.J. *et al.* (2004) J. Immunol. **172**:3406.
14. Bohlson, S.S. *et al.* (2005) J. Immunol. **175**:1239.
15. Greenlee, M.C. *et al.* (2009) Inflamm. Res. **58**:909.
16. Greenlee-Wacker, M.C. *et al.* (2011) J. Immunol. **187**:3353.
17. Yanaba, K. *et al.* (2012) Brit. J. Dermatol. **167**:542.
18. Jeon, J.W. *et al.* (2010) J. Immunol. **185**:4921.
19. Malarstig, A. *et al.* (2011) J. Intern. Med. **270**:229.
20. Nepomuceno, R.R. *et al.* (1999) J. Immunol. **162**:3583.
21. Kim, T.S. *et al.* (2000) Mol. Immunol. **37**:377.

©2012 R&D Systems, Inc.

10.12 752434.0 10/12