Quantikine® ELISA

Human C1q R1/CD93 Immunoassay

Catalog Number DCD930

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

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
MATERIALS PROVIDED & STORAGE CONDITIONS	3
OTHER SUPPLIES REQUIRED	3
PRECAUTIONS	4
SAMPLE COLLECTION & STORAGE	4
SAMPLE PREPARATION	4
REAGENT PREPARATION	5
ASSAY PROCEDURE	6
CALCULATION OF RESULTS	7
TYPICAL DATA	7
PRECISION	8
RECOVERY	8
SENSITIVITY	8
CALIBRATION	8
LINEARITY	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 is involved in various aspects of inflammatory reactions (1). Mature human CD93 consists of a 557 amino acid (aa) extracellular domain (ECD) with one C-type lectin domain, four 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, human CD93 shares 65% aa sequence identity with mouse and rat CD93. 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 (3, 5). CD93 is expressed by vascular endothelial cells (5, 6) 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 (3-5, 7-10). It is weakly expressed on resident tissue and peritoneal macrophages but is upregulated following inflammatory activation (5, 11).

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 (12-14). Soluble CD93 levels are elevated in peritoneal lavage fluid during peritonitis, in serum during sepsis, and in synovial fluid during rheumatoid arthritis relative to osteoarthritis (13-15). Moderate serum elevation of soluble CD93 is correlated with a decreased risk of coronary artery disease and myocardial infarction (16).

Cross-linking of cell surface CD93 enhances phagocytosis by monocytes and enhances the uptake of apoptotic cells *in vivo* (11, 17). Soluble CD93 binds directly to monocyte and vascular endothelial cell surfaces (5, 13). It promotes the differentiation of monocytes to macrophages, phagocytosis of apoptotic cells, and inflammatory responsiveness to multiple TLR ligands (13, 15). Although it contributes to these aspects of inflammation, CD93 is not required for macrophage recruitment during thioglycollate-induced peritonitis (11, 14). 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 (10).

The Quantikine Human C1q R1 Immunoassay is a 3.5 hour solid-phase ELISA designed to measure human C1q R1 in cell culture supernates, serum, plasma, saliva, and urine. It contains NS0-expressed recombinant human C1q R1 and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human C1q R1 showed linear curves that were parallel to the standard curves obtained using the Quantikine kit standards. These results indicate that this kit can be used to determine relative mass values for naturally occurring human C1q R1.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for C1q R1 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any C1q R1 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for 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 and color develops in proportion to the amount of C1q R1 bound in the initial step. The color development is stopped and the intensity of the color is measured.

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 ligands, binding proteins, and 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.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- 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. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

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
C1q R1 Microplate	894265	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against 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.*
C1q R1 Conjugate	894266	21 mL of polyclonal antibody against C1q R1 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
C1q R1 Standard	894267	40 ng of recombinant human C1q R1 in a buffered protein base with preservatives; lyophilized.	
Assay Diluent RD1-9	895167	11 mL of a buffered protein base with preservatives. *May contain a precipitate. Mix well before and during during use.*	
Calibrator Diluent RD5R	895190	2 vials (21 mL/vial) of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservatives.	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	6 mL of 2 N sulfuric 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.
- 500 mL graduated cylinder.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Test tubes for dilution of standards and samples.
- Human C1q R1 Controls (optional; available from R&D Systems).

PRECAUTIONS

C1q R1 is detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.

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

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 and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 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.*

Saliva - Collect saliva in a tube and centrifuge for 5 minutes at 10,000 x g. Collect the aqueous layer, and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, and assay immediately or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Serum and plasma samples require at least a 100-fold dilution. A suggested 100-fold dilution can be achieved by adding 10 µL of sample to 90 µL of Calibrator Diluent RD5R. Complete the 100-fold dilution by adding 20 µL of the diluted sample to 180 µL of Calibrator Diluent RD5R.

Saliva samples require a 2-fold dilution. A suggested 2-fold dilution is 75 µL of sample + 75 µL of Calibrator Diluent RD5R.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

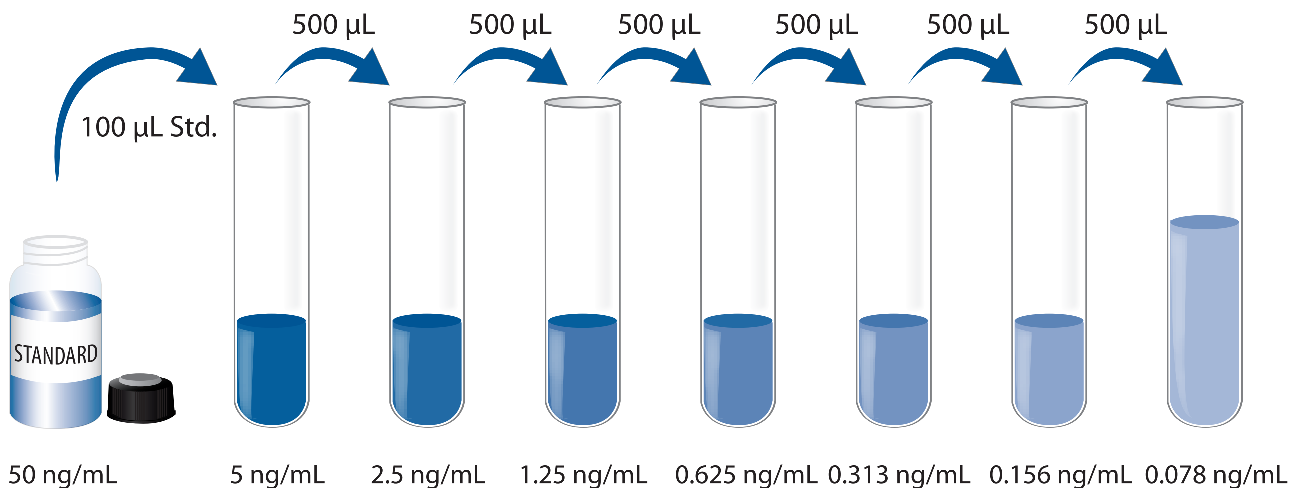
Note: *C1q R1 is detectable in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

C1q R1 Standard - Reconstitute the C1q R1 Standard with 1.0 mL of deionized or distilled water. This reconstitution produces a stock solution of 50 ng/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes. Mix well prior to making dilutions.

Pipette 900 µL of Calibrator Diluent RD5R into the 5 ng/mL tube. Pipette 500 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 5 ng/mL standard serves as the high standard. Calibrator Diluent RD5R serves as the zero standard (0 ng/mL).



ASSAY PROCEDURE

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

Note: *C1q R1 is detectable in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

1. Prepare all reagents, working standards, 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 100 µL of Assay Diluent RD1-9 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 on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.
5. Aspirate each well and wash, repeating the process three times for a total of four 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 200 µL of C1q R1 Conjugate to each well. Cover with a new adhesive strip. Incubate for 1 hour at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
9. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, 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

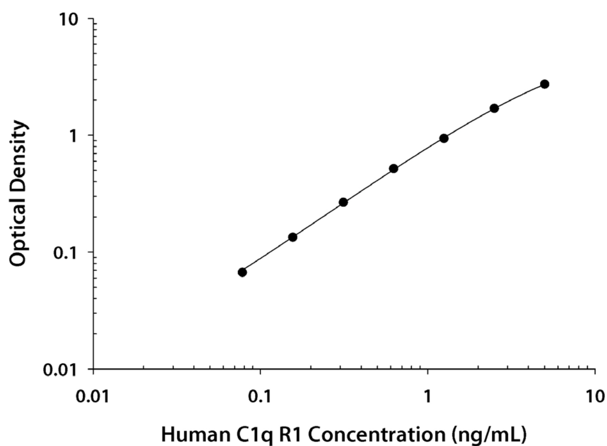
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density.

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 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, 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.



(ng/mL)	O.D.	Average	Corrected
0	0.010 0.010	0.010	—
0.078	0.075 0.078	0.077	0.067
0.156	0.141 0.146	0.144	0.134
0.313	0.271 0.280	0.276	0.266
0.625	0.524 0.530	0.527	0.517
1.25	0.945 0.948	0.947	0.937
2.5	1.695 1.714	1.705	1.695
5	2.713 2.764	2.739	2.729

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 separate assays to assess inter-assay precision.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (ng/mL)	0.49	1.58	3.41	0.48	1.51	3.19
Standard deviation	0.01	0.05	0.11	0.03	0.09	0.15
CV (%)	2.0	3.2	3.2	6.3	6.0	4.7

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture media (n=8)	99	92-108%
Serum* (n=4)	102	87-115%
EDTA plasma* (n=4)	104	94-118%
Heparin plasma* (n=4)	102	91-115%
Saliva (n=4)	104	94-112%
Urine (n=4)	97	93-102%

*Samples were diluted prior to assay.

SENSITIVITY

Twenty assays were evaluated and the minimum detectable dose (MDD) of C1q R1 ranged from 0.001-0.028 ng/mL. The mean MDD was 0.006 ng/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-expressed recombinant human C1q R1 produced at R&D Systems.

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of C1q R1 were serially diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=8)	Serum* (n=4)	EDTA plasma* (n=4)	Heparin plasma* (n=4)	Saliva (n=4)	Urine (n=4)
1:2	Average % of Expected	97	98	101	96	102	102
	Range (%)	93-102	96-101	97-108	90-102	101-102	101-103
1:4	Average % of Expected	97	97	100	100	102	99
	Range (%)	93-103	95-100	97-104	99-103	98-107	95-102
1:8	Average % of Expected	95	98	100	100	105	99
	Range (%)	89-99	95-101	94-108	97-105	101-108	95-102
1:16	Average % of Expected	92	98	98	100	100	98
	Range (%)	90-94	92-103	94-102	97-106	98-105	88-109

*Samples were diluted prior to assay.

SAMPLE VALUES

Serum/Plasma/Saliva/Urine - Samples from apparently healthy volunteers were evaluated for the presence of C1q R1 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=35)	146	90.9-223	32.9
EDTA plasma (n=35)	137	87.2-212	32.1
Heparin plasma (n=35)	144	78.0-268	38.2
Urine (n=12)	1.25	0.126-3.95	1.17

Sample Type	Mean of Detectable (ng/mL)	% Detectable	Range (ng/mL)
Saliva (n=8)	0.583	62	ND-2.13

ND=Non-detactable

Cell Culture Supernates:

HUVEC human umbilical vein endothelial cells were cultured in EGM supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate until confluent. An aliquot of the cell culture supernate was removed, assayed for levels of natural human C1q R1, and measured 1.48 ng/mL.

THP-1 human acute monocytic leukemia cells were cultured in RPMI-1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, and 50 µM β-mercaptoethanol. Cell were stimulated with 1 µg/mL lipopolysaccharide and grown until confluent. An aliquot of the cell culture supernate was removed, assayed for levels of natural human C1q R1, and measured 1.65 ng/mL.

SPECIFICITY

This assay recognizes natural and recombinant human C1q R1.

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range recombinant human C1q R1 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

Complement Component C5a
Complement Component C1r
Complement Component C1s
Complement Component C1rLP
Complement Component C1qA
Complement Component C1qC
Complement Component C3a
MBL

Recombinant mouse:

C1q R1/CD93

REFERENCES

1. Greenlee-Wacker, M.C. *et al*. (2012) Curr. Drug Targets **13**:411.
2. Nepomuceno, R.R. *et al*. (1997) Immunity **6**:119.
3. Steinberger, P. *et al*. (2002) J. Leukoc. Biol. **71**:133.
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. Xiao, S. *et al*. (2001) Clin. Exp. Immunol. **125**:360.
7. Lovik, G. *et al*. (2001) Scand. J. Immunol. **53**:410.
8. Danet, G.H. *et al*. (2002) Proc. Natl. Acad. Sci. USA **99**:10441.
9. Ikewaki, N. *et al*. (2010) J. Clin. Immunol. **30**:723.
10. Chevrier, S. *et al*. (2009) Proc. Natl. Acad. Sci. USA **106**:3895.
11. Norsworthy, P.J. *et al*. (2004) J. Immunol. **172**:3406.
12. Bohlson, S.S. *et al*. (2005) J. Immunol. **175**:1239.
13. Greenlee, M.C. *et al*. (2009) Inflamm. Res. **58**:909.
14. Greenlee-Wacker, M.C. *et al*. (2011) J. Immunol. **187**:3353.
15. Jeon, J.W. *et al*. (2010) J. Immunol. **185**:4921.
16. Malarstig, A. *et al*. (2011) J. Intern. Med. **270**:229.
17. Nepomuceno, R.R. *et al*. (1999) J. Immunol. **162**:3583.

©2012 R&D Systems, Inc.

06.12 752371.0 6/12