Quantikine® ELISA

Human FOLR1 Immunoassay

Catalog Number DFLR10

For the quantitative determination of human Folate Receptor 1 (FOLR1) concentrations in cell culture supernates, serum, plasma, saliva, urine, and human milk.

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
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

Folate Receptor 1 (FOLR1), also known as Folate Receptor alpha and Folate Binding Protein (FBP), is a 37-42 kDa protein that mediates the cellular uptake of folic acid and reduced folates. Dietary folates are required for many key metabolic processes including nucleotide and methionine synthesis, the interconversion of glycine and serine, and histidine breakdown (1, 2). Mature FOLR1 is an N-glycosylated protein that is anchored to the cell surface by a GPI linkage (3-6). Human FOLR1 shares 83% amino acid sequence identity with mouse and rat FOLR1. FOLR1 is predominantly expressed on epithelial cells and is dramatically upregulated on many carcinomas (7-9). It is critically required during early embryogenesis as shown in knockout mice which die in utero with gross morphological defects (10). FOLR1 is internalized to the endosomal system where it dissociates from its ligand before recycling to the cell surface (6, 11).

A soluble form of FOLR1 can be proteolytically shed from its GPI anchor (12, 13). It is found in serum (12, 14, 15), cord blood (16), urine (15), ovarian cyst fluid (17), breast milk (12, 18, 19), and semen (18). Serum FOLR1 levels are elevated in various conditions including folate or Vitamin B12 deficiency, pregnancy, liver disease, uremia, leukemia, and carcinoma (14, 20-22). FOLR1 levels are additionally elevated in the ascites and urine of carcinoma patients (15, 23).

The Quantikine Human FOLR1 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human FOLR1 in cell culture supernates, serum, plasma, saliva, urine, and human milk. It contains CHO cell-expressed recombinant human FOLR1 and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human FOLR1 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 FOLR1.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for FOLR1 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any FOLR1 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for FOLR1 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 FOLR1 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, 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.
- 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
FOLR1 Microplate	893972	96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse monoclonal antibody against FOLR1.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of zip-seal. May be stored for up to 1 month at 2-8 °C.*
FOLR1 Conjugate	893973	21 mL of polyclonal antibody against FOLR1 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
FOLR1 Standard	893974	30 ng of recombinant human FOLR1 in a buffer with preservatives; lyophilized.	
Assay Diluent RD1-27	895245	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD6-9	895423	21 mL 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.
- 100 mL and 500 mL graduated cylinders.
- Test tubes for dilution of standards and samples.
- Human FOLR1 Controls (optional; available from R&D Systems).

PRECAUTIONS

FOLR1 is detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.

Calibrator Diluent RD6-9 contains sodium azide, which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

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

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 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.

Human Milk - Centrifuge for 15 minutes at 10,000 x g at 2-8 °C. Collect the aqueous fraction and centrifuge twice more for a total of 3 times. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Saliva samples require at least a 50-fold dilution. A suggested 50-fold dilution is 10 μL of sample + 490 μL of Calibrator Diluent RD6-9 (1:2).

Urine samples require at least a 10-fold dilution. A suggested 10-fold dilution is 20 μL of sample + 180 μL of Calibrator Diluent RD6-9 (1:2).

Human milk samples require at least a 100-fold dilution. A suggested 100-fold dilution is 10 μL of sample + 490 μL of Calibrator Diluent RD6-9 (1:2). Complete the 100-fold dilution by adding 75 μL of this solution to 75 μL of Calibrator Diluent RD6-9 (1:2).

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Note: *FOLR1 is detectable in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

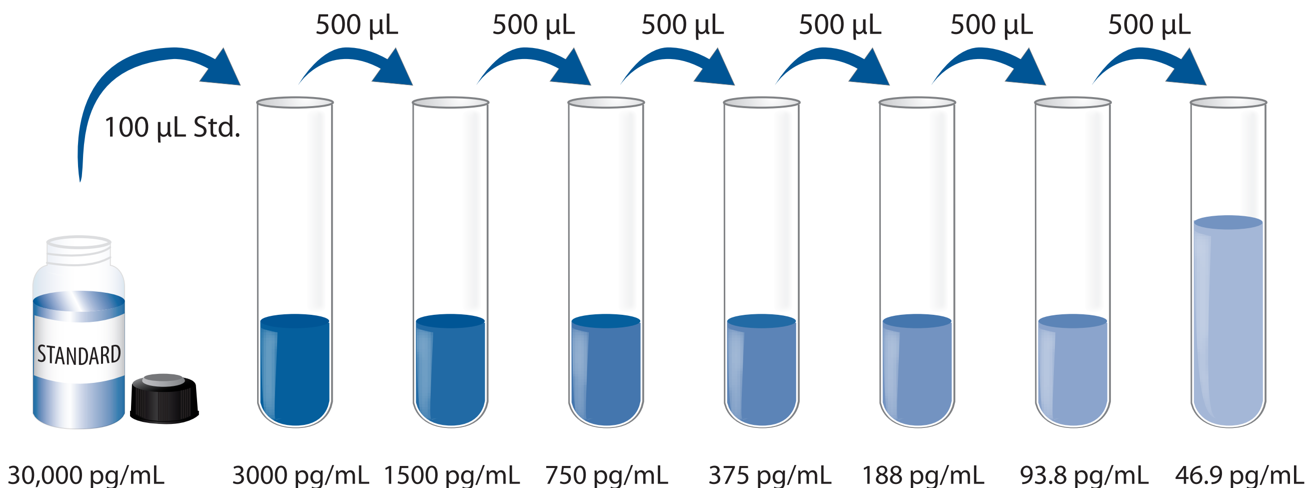
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 500 mL of Wash Buffer.

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.

Calibrator Diluent RD6-9 (1:2) - Dilute 20 mL of Calibrator Diluent RD6-9 into 20 mL of deionized or distilled water to prepare 40 mL of Calibrator Diluent RD6-9 (1:2).

FOLR1 Standard - Reconstitute the FOLR1 Standard with 1.0 mL of deionized or distilled water. This reconstitution produces a stock solution of 30,000 pg/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 RD6-9 (1:2) into the 3000 pg/mL tube. Pipette 500 µL of Calibrator Diluent RD6-9 (1:2) into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 3000 pg/mL standard serves as the high standard. Calibrator Diluent RD6-9 (1:2) 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, samples, and controls be assayed in duplicate.

Note: *FOLR1 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 RD1-27 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 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 FOLR1 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.
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. **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

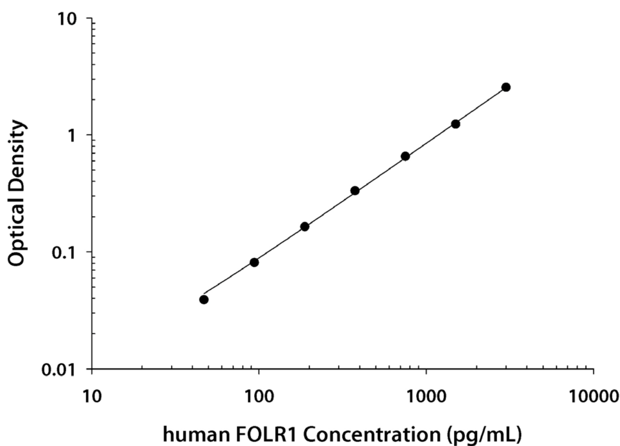
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density.

Create a standard curve by reducing the data using computer software capable of generating a log/log 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 a log/log graph. The data may be linearized by plotting the log of the FOLR1 concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

If the 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.



(pg/mL)	O.D.	Average	Corrected
0	0.014 0.014	0.014	—
46.9	0.052 0.053	0.053	0.039
93.8	0.094 0.095	0.095	0.081
188	0.175 0.180	0.178	0.164
375	0.336 0.358	0.347	0.333
750	0.668 0.669	0.669	0.655
1500	1.243 1.253	1.248	1.234
3000	2.547 2.573	2.560	2.546

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 (pg/mL)	377	1024	1922	390	1050	1973
Standard deviation	9.28	19.8	29.5	20.3	64.0	135
CV (%)	2.5	1.9	1.5	5.2	6.1	6.9

RECOVERY

The recovery of FOLR1 spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	98	94-107%
Serum (n=4)	96	84-107%
EDTA plasma (n=4)	95	84-106%
Heparin plasma (n=4)	96	82-105%

LINEARITY

To assess the linearity of the assay, samples containing high concentrations of FOLR1 were serially diluted with Calibrator Diluent RD6-9 (1:2) to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=4)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)	Saliva* (n=4)	Urine* (n=4)	Human milk* (n=4)
1:2	Average % of Expected	103	101	101	101	102	101	101
	Range (%)	102-104	95-105	96-104	97-104	100-104	94-107	98-102
1:4	Average % of Expected	96	103	101	102	102	101	100
	Range (%)	93-99	100-105	96-106	98-107	100-103	91-109	99-102
1:8	Average % of Expected	102	105	98	96	100	100	98
	Range (%)	100-105	102-106	90-104	91-102	99-103	90-110	96-100
1:16	Average % of Expected	94	101	96	92	99	98	93
	Range (%)	——	94-109	89-103	85-101	96-103	87-105	89-98

*Samples were diluted prior to assay.

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

SENSITIVITY

Thirty assays were evaluated and the minimum detectable dose (MDD) of FOLR1 ranged from 1.67-14.9 pg/mL. The mean MDD was 5.54 pg/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 CHO cell-expressed recombinant human FOLR1 produced at R&D Systems.

SAMPLE VALUES

Serum/Plasma/Saliva/Urine/Human Milk - Samples from apparently healthy volunteers were evaluated for the presence of FOLR1 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=35)	710	427-1034	130
EDTA plasma (n=35)	676	410-967	124
Heparin plasma (n=35)	725	382-1020	145
Saliva (n=10)	49,464	2350-163,200	60,470
Urine (n=10)	13,930	2896-25,990	7302
Human milk (n=10)	807,260	438,800-1,509,600	347,670

Cell Culture Supernates -
A431 human epithelial carcinoma cells were cultured in DMEM 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 FOLR1, and measured 220 pg/mL.

SPECIFICITY

This assay recognizes recombinant and natural human FOLR1. 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 FOLR1 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:	Recombinant mouse:
FOLR2	FOLR1
FOLR3	FOLR2
FOLR4	FOLR4

This kit recognizes recombinant human FOLR1 in the presence of folic acid.

REFERENCES

1. Kelemen, L.E. (2006) Int. J. Cancer **119**:243.
2. Fowler, B. *et al.* (2001) Kidney Int. **59**:S221.
3. Luhrs, C.A. *et al.* (1989) J. Biol. Chem. **264**:21446.
4. Lacey, S.W. *et al.* (1989) J. Clin. Invest. **84**:715.
5. Elwood, P.C. (1989) J. Biol. Chem. **264**:14893.
6. Rijnboutt, S. *et al.* (1996) J. Cell Biol. **132**:35.
7. Ross, J.F. *et al.* (1994) Cancer **73**:2432.
8. Parker, N. *et al.* (2005) Anal. Biochem. **338**:284.
9. Clifton, G.T. *et al.* (2011) Hum. Vaccin. **7**:183.
10. Piedrahita, J.A. *et al.* (1999) Nat. Genet. **23**:228.
11. Paulos, C.M. *et al.* (2004) Mol. Pharmacol. **66**:1406.
12. Elwood, P.C. *et al.* (1991) J. Biol. Chem. **26**:2346.
13. Verma, R.S. and A.C. Antony (1991) J. Biol. Chem. **266**:12522.
14. da Costa, M. *et al.* (1978) J. Lab. Clin. Med. **91**:901.
15. Mantovani, L.T. *et al.* (1994) Eur. J. Cancer **30A**:363.
16. Kamen, B.A. and J.D. Caston (1975) Proc. Natl. Acad. Sci. **72**:4261.
17. Holm, J. *et al.* (1995) APMIS **103**:862.
18. Hansen, S.I. and J. Holm (1992) Biosci. Rep. **12**:87.
19. Antony, A.C. *et al.* (1982) J. Biol. Chem. **257**:10081.
20. da Costa, M. and C. Fisher (1981) J. Lab. Clin. Med. **98**:956.
21. Basal, E. *et al.* (2009) PLoS ONE **4**:e6292.
22. Waxman, X. and C. Schreiber (1973) Blood **42**:291.
23. Holm, J. *et al.* (1999) Arch. Biochem. Biophys. **366**:183.

©2011 R&D Systems, Inc.

09.11 752173.0 9/11

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