

Quantikine[®] ELISA

Mouse IL-34 Immunoassay

Catalog Number M3400

For the quantitative determination of mouse Interleukin 34 (IL-34) 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
MATERIALS PROVIDED & STORAGE CONDITIONS	3
OTHER SUPPLIES REQUIRED	4
OTHER SUPPLIES REQUIRED FOR TISSUE LYSATE SAMPLES	4
PRECAUTIONS	4
SAMPLE COLLECTION & STORAGE	5
REAGENT PREPARATION	6
ASSAY PROCEDURE	7
CALCULATION OF RESULTS	8
TYPICAL DATA	8
PRECISION	9
RECOVERY	9
LINEARITY	9
SENSITIVITY	10
CALIBRATION	10
SAMPLE VALUES	10
SPECIFICITY	11
REFERENCES	11
PLATE LAYOUT	12

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

Interleukin 34 (IL-34) is a 39-45 kDa member of the short chain α -helix cytokine family of molecules (1-3). It is secreted as a variably glycosylated, noncovalent homodimer whose biology generally parallels that of M-CSF/CSF-1 (1, 2, 4-6). A splice form lacking Gln81, and another with alternate sequence replacing amino acid (aa) 135-235 (C-terminus), have been described (7). The isoform lacking Gln81 has less potency than the Gln-containing isoform on macrophage activity (8). Mature mouse IL-34 shares 69% aa sequence identity with human IL-34 (1, 8). This modest orthology reflects cross-species activity where human IL-34 shows limited activity on mouse cells, and mouse IL-34 shows almost no activity on human cells (9). IL-34 is secreted by multiple cell types, including synovial fibroblasts (10), neurons and keratinocytes (11-13), splenic sinusoidal endothelial cells (1, 14), osteoblasts (8, 15), and possibly osteoclasts (16).

IL-34 has multiple functions associated with it, some of which parallel those of M-CSF. In particular, IL-34 will cooperate with RANKL to generate osteoclasts from osteoclast precursors in the spleen (15-17). IL-34 will also substitute for M-CSF in the generation of macrophages from monocytes (1, 2, 4, 6). This overlap is possible because IL-34 and M-CSF share the same receptor, M-CSF R/CSF1R (1, 2, 4, 5). Notably, the binding characteristics are distinct; IL-34 and M-CSF do not bind to the same motif, and they do not bind with the same efficacy. For example, IL-34's interaction with M-CSF R is hydrophobic and extended, while M-CSF's interaction is hydrophilic and abbreviated (5, 6). These differences may partially account for distinct IL-34 activities, among which are the generation of macrophages that express high amounts of Eotaxin-2 and low amounts of MCP-1, and the maintenance of skin Langerhans cell numbers at levels necessary for homeostatic monitoring (6, 11). Finally, IL-34 has been suggested to play a key role in brain development and homeostasis (18). In particular, IL-34 is reported to induce neuronal differentiation, an activity shared with M-CSF. The expression patterns of both secreted ligands are spatially and temporally distinct, and the appearance of their common receptor is not correlated with their expression. This suggests that in the brain, M-CSF and IL-34 are not redundant, and IL-34 is proposed to bind and signal through an additional receptor in mouse brain, protein tyrosine phosphatase zeta (PTP- ζ), in a chondroitin sulfate-dependent manner (18, 19). IL-34 is also known to induce the secretion of TGF- β , HO-1 (heme oxygenase-1), and IDE (insulin degrading enzyme) from microglia, three molecules which have the potential to serve as a first-line defense against soluble oligomeric A β , one of the initiating factors in Alzheimer's disease (13, 20).

The Quantikine[®] Mouse IL-34 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure mouse IL-34 in cell culture supernates, tissue lysates, serum, and plasma. It contains NS0-expressed recombinant mouse IL-34 and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate the recombinant mouse IL-34. Results obtained using natural mouse IL-34 showed dose response 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 natural mouse IL-34.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse IL-34 has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any IL-34 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for mouse IL-34 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 IL-34 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, dilute samples with calibrator diluent and repeat the assay.
- Any variation in 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.

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.
- 50 mL and 500 mL graduated cylinders.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Test tubes for dilution of standards.

OTHER SUPPLIES REQUIRED FOR TISSUE LYSATE SAMPLES

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

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the SDS on our website prior to use.

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, tissue 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: *Variations in sample collection, processing, and storage may cause sample value differences. Adhere to consistent 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.
Grossly icteric samples are not suitable for use in this assay.*

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Mouse IL-34 Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

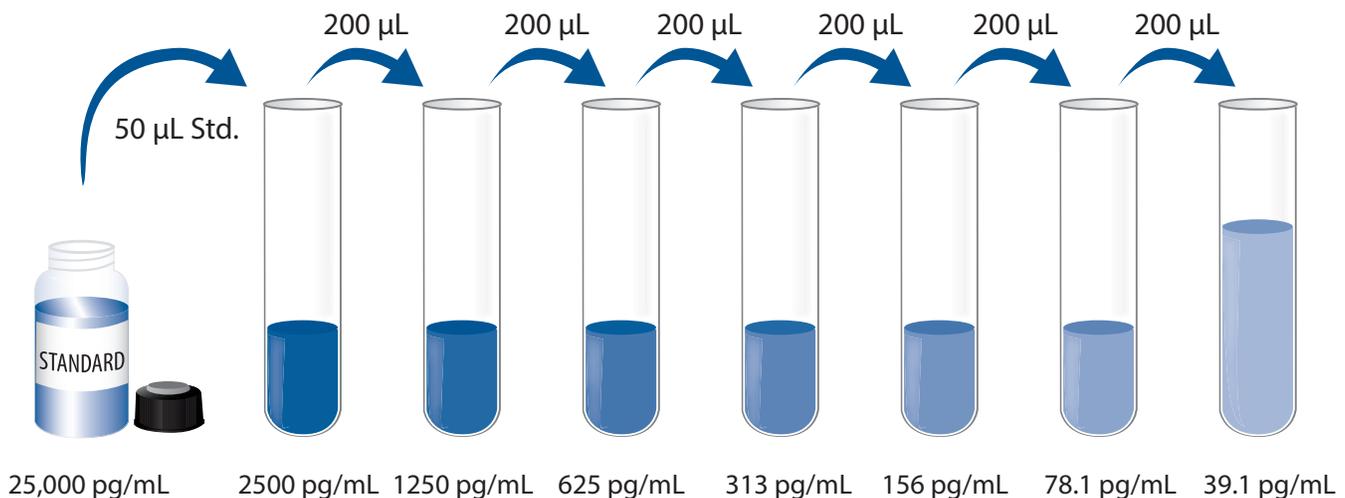
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

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

Calibrator Diluent RD5P (diluted 1:5) - Add 10 mL of Calibrator Diluent RD5P to 40 mL of deionized or distilled water to prepare 50 mL of Calibrator Diluent RD5P (diluted 1:5).

Mouse IL-34 Standard - Refer to the vial label for reconstitution volume. Reconstitute the Mouse IL-34 Standard with deionized or distilled water. This reconstitution produces a stock solution of 25,000 pg/mL. Allow the stock solution to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Pipette 450 μ L of Calibrator Diluent RD5P (diluted 1:5) into the 2500 pg/mL tube. Pipette 200 μ L into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 2500 pg/mL standard serves as the high standard. Calibrator Diluent RD5P (diluted 1:5) 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, standard dilutions, 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 μ L of Assay Diluent RD1-63 to each well.
4. Add 50 μ L 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. A plate layout is provided to record standards and samples assayed.
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 μ L) 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 μ L of Mouse IL-34 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 μ L of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
9. Add 100 μ L 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.

CALCULATION OF RESULTS

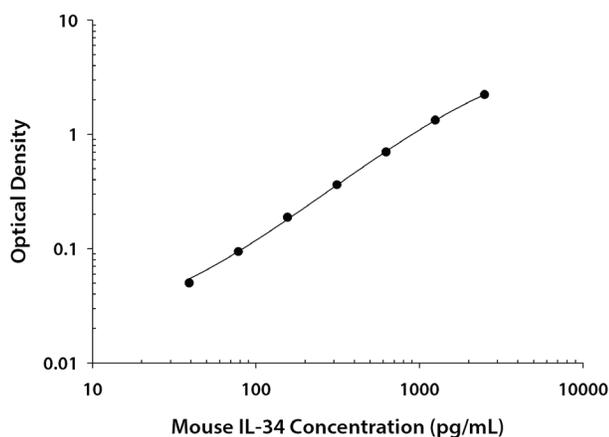
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 IL-34 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.



(pg/mL)	O.D.	Average	Corrected
0	0.013 0.014	0.014	—
39.1	0.063 0.065	0.064	0.050
78.1	0.107 0.109	0.108	0.094
156	0.201 0.203	0.202	0.188
313	0.372 0.380	0.376	0.362
625	0.702 0.727	0.715	0.701
1250	1.333 1.354	1.344	1.330
2500	2.210 2.263	2.237	2.223

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. Assays were performed by at least three technicians using two lots of kit components.

Sample	Intra-Assay Precision			Inter-Assay Precision		
	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	99.6	273	870	107	284	903
Standard deviation	2.4	4.0	12.7	8.5	15.5	33.3
CV (%)	2.4	1.5	1.5	7.9	5.5	3.7

RECOVERY

The recovery of mouse IL-34 spiked to levels throughout the range of the assay in various matrices was evaluated.

	Average % Recovery	Range
Cell culture media (n=4)	98	92-103%
Serum (n=4)	100	95-107%
EDTA plasma (n=4)	100	85-109%
Heparin plasma (n=4)	103	99-110%

LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of mouse IL-34 in each matrix were serially diluted with calibrator diluent 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)
1:2	Average % of Expected	101	104	106	102
	Range (%)	97-104	101-105	99-118	98-104
1:4	Average % of Expected	98	105	108	100
	Range (%)	96-101	101-109	98-115	99-101
1:8	Average % of Expected	97	103	102	96
	Range (%)	94-102	100-105	96-113	93-97
1:16	Average % of Expected	91	102	99	96
	Range (%)	86-97	98-105	94-104	92-102

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of mouse IL-34 ranged from 1.54-15.6 pg/mL. The mean MDD was 5.5 pg/mL.

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

CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant mouse IL-34 produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Samples were evaluated for the presence of mouse IL-34 in this assay.

Samples	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=10)	66.8	80	ND-81.6
EDTA plasma (n=5)	72.8	20	ND-72.8
Heparin plasma (n=5)	47.8	20	ND-47.8

ND=Non-detectable

Cell Culture Supernates - Brains from mice were rinsed with PBS then homogenized with 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 for 3 days. Cells were cultured unstimulated or stimulated with 1.0 µg/mL of LPS or 1.0 µg/mL of recombinant human (rh) IL-2. Aliquots of the cell culture supernates were removed and assayed for mouse IL-34.

Condition	(pg/mL)
Unstimulated	ND
Stimulated w/LPS	50.7
Stimulated w/rhIL-2	144

ND=Non-detectable

Tissue Lysates - Brains from mice were rinsed with PBS, cut into 1-2 mm pieces, 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. An aliquot of the lysate was removed, assayed for mouse IL-34, and measured 955 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant mouse IL-34.

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 mouse IL-34 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:

M-CSF

M-CSF R

TRANCE/RANKL

Recombinant human:

IL-34

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PLATE LAYOUT

Use this plate layout to record standards and samples assayed.

12									
11									
10									
9									
8									
7									
6									
5									
4									
3									
2									
1									
	A	B	C	D	E	F	G	H	

NOTES

NOTES

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