Quantikine® ELISA

Human IL-34 Immunoassay

Catalog Number D3400

For the quantitative determination of human Interleukin 34 (IL-34) concentrations in cell culture supernates, serum, plasma, saliva, 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

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INTRODUCTION

Interleukin 34 (IL-34), also known as C16orf77, 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). Human IL-34 is synthesized as a 242 amino acid (aa) precursor that contains a 20 aa signal sequence plus a 222 aa mature segment (1, 7). The mature region contains up to six α-helices plus a C-terminal Pro-rich region (aa 194-224) (5, 7). One splice form is known that shows a deletion of Gln81. This isoform has less potency than the Gln-containing isoform on macrophage activity (8). Mature human IL-34 shares 69% and 73% aa sequence identity with mouse and porcine IL-34, respectively (1, 8). This modest orthology reflects cross-species activity where human IL-34 shows limited activity on mouse and porcine cells, mouse IL-34 shows almost no activity on human cells, and porcine IL-34 is active on both mouse and 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. As noted, some of its actions parallel those of M-CSF. In particular, and as for M-CSF, 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 (or theoretical redundancy) 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 high expressing eotaxin-2/low expressing MCP-1 macrophages, and the maintenance of skin Langerhans cell numbers at levels necessary for homeostatic monitoring (6, 11). Finally, IL-34 has recently been suggested to play a key role in brain development and homeostasis. In particular, IL-34 is reported to induce neuronal differentiation, an activity not unexpectedly shared with M-CSF. But of note, 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 that an additional receptor may exist for IL-34 (18). In addition, IL-34 is 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 Aβ, one of the initiating factors in Alzheimer's disease (13, 19).

The Quantikine® Human IL-34 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human IL-34 in cell culture supernates, serum, plasma, saliva, and human milk. It contains NS0-expressed recombinant human IL-34 and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human IL-34 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 natural human IL-34.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human IL-34 has been pre-coated onto a microplate. Standards 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 polyclonal antibody specific for human 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 and color develops in proportion to the amount of IL-34 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.
- 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.
- 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
Human IL-34 Microplate	894549	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human IL-34.	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.*
Human IL-34 Conjugate	894550	21 mL of a polyclonal antibody specific for human IL-34 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human IL-34 Standard	894551	Recombinant human IL-34 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent RD1-77	895545	17 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5P Concentrate	895151	21 mL of a concentrated buffered protein base with preservatives. *Use diluted 1:5 in this assay.*	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
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.
- 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 and samples.
- Human IL-34 Controls (optional; R&D Systems®, Catalog # QC31).

PRECAUTIONS

IL-34 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.

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 MSDS 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 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 EDTA or heparin 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.

Human Milk - Centrifuge for 15 minutes at 1000 x g at 2-8 °C. Collect the aqueous fraction and repeat this process a total of 3 times. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Human milk samples require at least a 4-fold dilution. A suggested 4-fold dilution is 50 µL of sample + 150 µL of Calibrator Diluent RD5P (diluted 1:5)*.

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Note: *High concentrations of IL-34 are found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

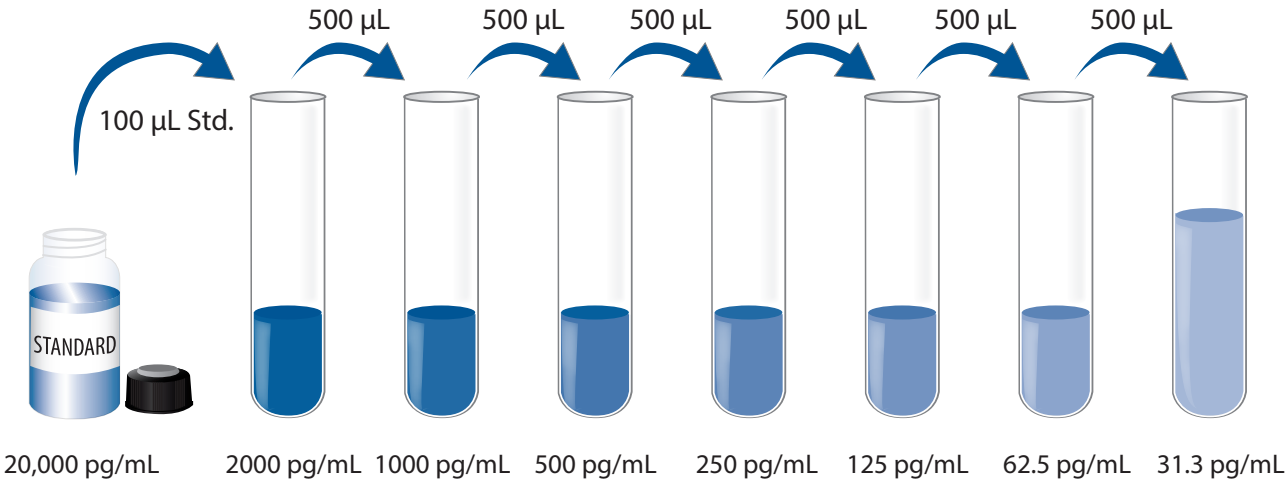
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

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 RD5P (diluted 1:5) - Add 3 mL of Calibrator Diluent RD5P Concentrate to 12 mL of deionized or distilled water to prepare 15 mL of Calibrator Diluent RD5P (diluted 1:5).

Human IL-34 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human IL-34 Standard with deionized or distilled water. This reconstitution produces a stock solution of 20,000 pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 900 µL of Calibrator Diluent RD5P (diluted 1:5) into the 2000 pg/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 2000 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 samples, controls, and standards be assayed in duplicate.

Note: *High concentrations of IL-34 are found 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 RD1-77 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 Human 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 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 the Sample Preparation section.

CALCULATION OF RESULTS

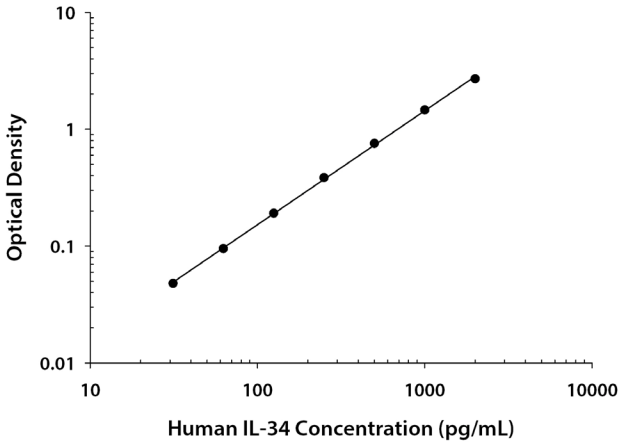
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a 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 human IL-34 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	0.017 0.017	0.017	—
31.3	0.061 0.068	0.065	0.048
62.5	0.109 0.114	0.112	0.095
125	0.205 0.210	0.208	0.191
250	0.394 0.410	0.402	0.385
500	0.755 0.790	0.773	0.756
1000	1.455 1.499	1.477	1.460
2000	2.695 2.733	2.714	2.697

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 components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	20	20	20
Mean (pg/mL)	212	571	1174	215	597	1206
Standard deviation	15.5	14.4	21.2	12.8	35.4	50.0
CV (%)	7.3	2.5	1.8	6.0	5.9	4.1

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	97	85-104%
Serum (n=4)	98	90-106%
EDTA plasma (n=4)	97	90-102%
Heparin plasma (n=4)	97	88-105%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human IL-34 were 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)	Human milk* (n=4)
1:2	Average % of Expected	104	102	102	104	105
	Range (%)	102-105	99-105	99-105	101-105	101-107
1:4	Average % of Expected	106	104	107	106	106
	Range (%)	102-109	100-109	101-114	101-110	99-111
1:8	Average % of Expected	106	104	106	108	105
	Range (%)	100-109	100-110	100-114	102-111	102-109
1:16	Average % of Expected	104	99	104	104	101
	Range (%)	96-112	92-106	97-116	99-110	92-107

*Samples were diluted prior to assay.

SENSITIVITY

Twenty-seven assays were evaluated and the minimum detectable dose (MDD) of human IL-34 ranged from 0.870-3.06 pg/mL. The mean MDD was 1.78 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 human IL-34 manufactured at R&D Systems®.

SAMPLE VALUES

Serum/Plasma/Saliva/Human Milk - Thirty-five serum and plasma, eleven saliva, and eight human milk samples from apparently healthy volunteers were evaluated for the presence of human IL-34 in this assay. No detectable levels were observed for serum or plasma. No medical histories were available for the donors used in this study.

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Saliva (n=11)	84.0	82	ND-126

ND=Non-detectable

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Human milk (n=8)	2877	657-9185	2768

Cell Culture Supernates - HUVEC human umbilical vein endothelial cells were cultured in EGM-2 to 80% confluency. Cells were stimulated with 40 ng/mL of recombinant human TNF-α and 50 ng/mL of recombinant human IFN-γ for 2 days. An aliquot of the cell culture supernate was removed, assayed for human IL-34, and measured 38.0 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant human 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 recombinant human IL-34 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:	Recombinant mouse:
M-CSF	IL-34
	M-CSF
	M-CSF R

Recombinant human M-CSF R interferes at concentrations > 5 ng/mL in this assay.

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