Quantikine® ELISA

Human IL-33 Immunoassay

Catalog Number D3300B

For the quantitative determination of human Interleukin 33 (IL-33) concentrations in cell culture supernates, cell lysates, 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.

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INTRODUCTION

Interleukin-33 (IL-33), also known as NF-HEV and DVS 27, is a 30 kDa pro-inflammatory protein that plays an important role in Th2-biased immune responses and cardiac pathology (1-3). Human IL-33 is synthesized as a 270 amino acid (aa) molecule with an N-terminal nuclear localization signal, a helix-turn-helix motif, and a C-terminal region with structural homology to IL-1 family cytokines (4). Full length IL-33 interacts with nuclear chromatin, binds NFκB, and inhibits pro-inflammatory NFκB transactivation (5-7). Cleavage of full length IL-33 leads to the extracellular release of an 18-20 kDa C-terminal fragment known as mature IL-33 (4, 8). Cathepsin G, Elastase, and Proteinase 3 can each cleave full length IL-33, giving rise to N-terminal heterogeneity of the mature form (9, 10). IL-33 can be inactivated by further cleavage at several sites by Proteinase 3 and Caspase-1 (10, 11). Additional isoforms of human IL-33 with internal deletions are generated by alternative splicing (12, 13). Mature human IL-33 shares 57% and 59% aa sequence identity with mouse and rat IL-33, respectively.

IL-33 binds the transmembrane receptor ST2/IL-1 R4 which subsequently associates with IL-1 RAcP to enable IL-33 dependent signaling (4, 14-16). IL-1 RAcP is a shared signaling subunit that also associates with the receptors IL-1 RI, IL-1 RII, IL-1 R6, and SCF R/c-kit (17). A soluble isoform of ST2 retains the ability to bind IL-33 and blocks ST2-dependent responses (18, 19). Soluble IL-1 RAcP enhances the decoy function of soluble ST2 (16). IL-33 binding to transmembrane ST2 induces the association of ST2 with existing IL-1 RAcP/SCF R complexes (20). Activation of either ST2 or SCF R by their respective ligands can induce signal transduction through the other receptor subunit (20). IL-33 signaling through ST2 additionally triggers VE-Cadherin phosphorylation and internalization on vascular endothelial cells which leads to increased vascular permeability, vessel sprouting, and tubule formation (21).

IL-33 exerts multiple effects on immune system function. It acts on Th2 cells, basophils, and mast cells to induce their migration to sites of inflammation and production of Th2 cytokines (4, 22-26). IL-33 also promotes the expansion of regulatory T cells and alternately activated macrophages while attenuating Th17 cell expansion and activation (27). IL-33 contributes to infection clearance by enhancing neutrophil sensitization to TLR and Dectin-1 signaling, phagocytic activity, and migration to sites of infection (22, 28, 29). It is upregulated in a wide variety of cells under inflammatory conditions (5, 30, 31). Full length IL-33 is also found at elevated levels in bronchiolar lavage fluid during pulmonary fibrosis (32). The full length protein is classified as an alarmin due to its release from physically damaged or necrotic cells and its ability to trigger inflammatory and anti-viral $CD8^+$ T cell responses (11, 33). Like mature IL-33, the full length protein activates ST2 and promotes mast cell activation and neutrophil infiltration (8, 9, 11, 32).

IL-33 induces both protective and pathologic actions in the heart. It counteracts cardiac myocyte hypertrophy and responsiveness to angiotensin II and phenylephrine (18, 34). It is induced in cardiac fibroblasts by mechanical stress and circulates at elevated levels during chronic heart failure (as does the full length form) (18, 35). The soluble ST2 receptor is elevated in the serum of heart failure as well as asthma patients (18, 19). IL-33 inhibits the development of atherosclerotic plaques and induces the production of anti-oxidized LDL antibodies (23). It can also enhance eosinophilic perimyocarditis and impair heart function (34). In other settings, IL-33 limits neutrophil infiltration and circulating inflammatory chemokine levels following hepatic ischemia/reperfusion injury (36) but exacerbates $CD4^+$ T cell infiltration and tissue damage following cisplatin-induced acute kidney injury (37).

The Quantikine® Human IL-33 Immunoassay is a 4.0 hour solid phase ELISA designed to measure human IL-33 in cell culture supernates, cell lysates, tissue lysates, serum, and plasma. It contains *E. coli*-expressed recombinant human IL-33 and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate recombinant human IL-33. Results obtained using natural human IL-33 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-33.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human IL-33 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-33 present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated monoclonal antibody specific for human IL-33 is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, an enzyme-linked streptavidin is added to the wells. After washing away any unbound streptavidin-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of IL-33 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 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-33 Microplate	898791	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human IL-33.	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-33 Standard	898793	2 vials of recombinant human IL-33 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Use a new standard for each assay. Discard after use.
Human IL-33 Conjugate	898792	21 mL of a monoclonal antibody specific for human IL-33 conjugated to biotin with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Streptavidin-HRP 1	898926	21 mL of a solution with preservatives.	
Assay Diluent RD1-63	895352	12 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5P	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.
- PBS (for cell lysates)
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Test tubes for dilution of standards.
- Human IL-33 controls (optional; R&D Systems®, Catalog # QC244).

SUPPLIES REQUIRED FOR CELL LYSATE SAMPLES

- Lysis Buffer 17 (R&D Systems®, Catalog # 895943)
- PBS

SUPPLIES REQUIRED FOR TISSUE LYSATE SAMPLES

- RIPA Buffer with protease inhibitors

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

Cell Lysates - Lysates were prepared as described in the Sample Values section.

Tissue Lysates - Lysates were prepared as described in the Sample Values section.

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.*

SAMPLE PREPARATION

Cell lysates utilizing a RIPA buffer require an initial 40-fold dilution due to a matrix effect. The suggested range for total cell lysate protein added is 3-50 µg/well.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

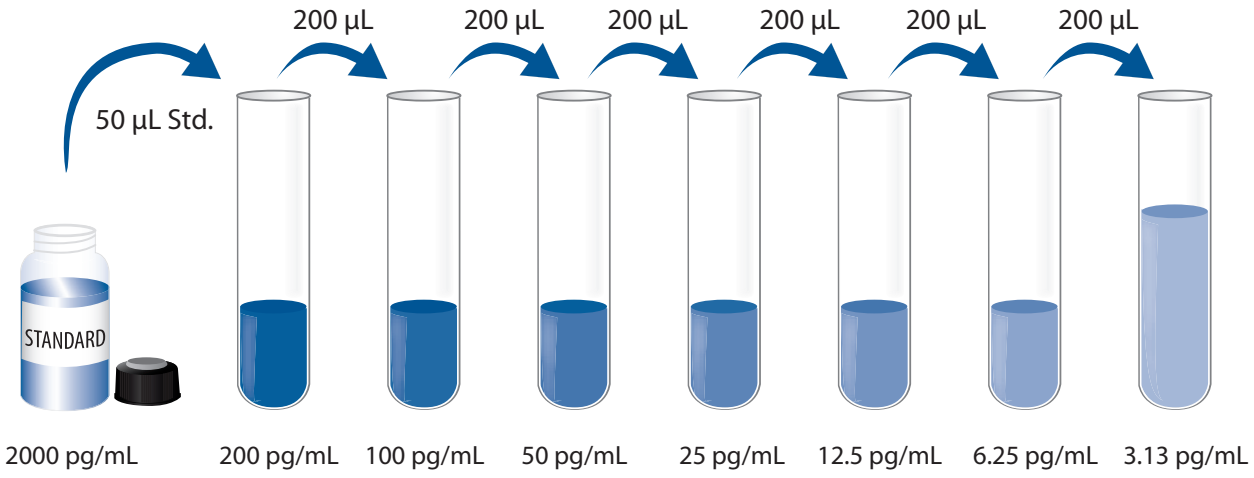
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL 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).

Human IL-33 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human IL-33 Standard with deionized or distilled water. This reconstitution produces a stock solution of 2000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 450 µL of Calibrator Diluent RD5P (diluted 1:5) into the 200 pg/mL tube. Pipette 200 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 200 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, samples, and controls be assayed in duplicate.

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 50 µL of Assay Diluent RD1-63 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 rpm ± 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 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 toweling.
6. Add 200 µL of Human IL-33 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 Streptavidin-HRP 1 to each well. Cover with a new adhesive strip. Incubate for 30 minutes at room temperature on the shaker.
9. Repeat the aspiration/wash as in step 5.
10. Add 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
11. Add 50 µL of Stop Solution to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. 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

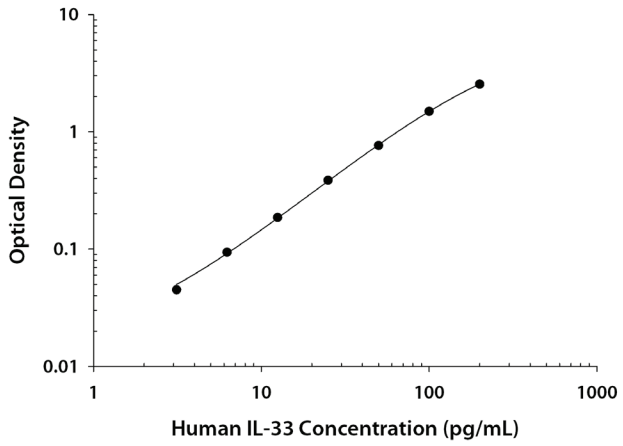
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 human IL-33 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	0.019 0.019	0.019	—
3.13	0.063 0.065	0.064	0.045
6.25	0.111 0.114	0.113	0.094
12.5	0.204 0.205	0.205	0.186
25	0.405 0.407	0.406	0.387
50	0.772 0.797	0.785	0.766
100	1.514 1.516	1.515	1.496
200	2.531 2.606	2.569	2.550

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 over one lot of kit components. Assays were performed by at least three technicians.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	20	20	20
Mean (pg/mL)	31.0	59.8	119	31.0	59.5	117
Standard deviation	1.15	2.72	6.99	1.35	2.60	6.98
CV (%)	3.7	4.5	5.9	4.4	4.4	6.0

RECOVERY

The recovery of human IL-33 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	88-107%
Lysis buffer (n=4)	103	86-123%
Extraction buffer (1X) (n=2)	103	81-115%
Serum (n=4)	89	85-96%
EDTA plasma (n=4)	91	83-103%
Heparin plasma (n=4)	89	85-95%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human IL-33 were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=4)	Lysis buffer (n=4)	Tissue lysates (n=2)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)
1:2	Average % of Expected	106	105	101	104	102	105
	Range (%)	102-109	102-108	99-103	98-108	98-105	103-108
1:4	Average % of Expected	110	102	105	107	105	110
	Range (%)	104-114	94-108	105-106	98-113	100-110	102-116
1:8	Average % of Expected	114	97	107	113	109	110
	Range (%)	110-118	86-105	105-110	103-118	97-117	92-120
1:16	Average % of Expected	116	85	109	111	108	115
	Range (%)	114-119	78-93	103-116	105-115	102-112	109-123

*Sample were diluted prior to assay.

SENSITIVITY

Sixteen assays were evaluated and the minimum detectable dose (MDD) of human IL-33 ranged from 0.069-1.51 pg/mL. The mean MDD was 0.357 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 *E. coli*-expressed recombinant human IL-33 produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Thirty samples from apparently healthy volunteers were evaluated for the presence of human IL-33 in this assay. No detectable levels were observed. No medical histories were available for the donors used in this study.

Cell Culture Supernates/Cell Lysates:
DLD-1 human colorectal adenocarcinoma leukemia cells were cultured in RPMI supplemented with 10% fetal bovine serum until 90% confluent. Cells were left untreated, treated for 4 hours with 1.0 µg/mL LPS, or treated for 4 hours with 1.0 µg/mL LPS followed by 1 hour with 5 mM ATP prior to collecting cells and cell culture conditioned media supernate.

For cell culture supernates, DLD-1 cells were left untreated or treated with LPS + ATP. The cells along with cell culture conditioned media supernate were centrifuged at 500 x g for 5 minutes. Aliquots of the cell culture supernates were removed and assayed for human IL-33.

Condition	(pg/mL)
Untreated	16.7
Treated	7.65

For cell lysates, DLD-1 cells were left untreated or treated with LPS. The cells were solubilized in Lysis Buffer 17 and allowed to sit on ice for 30 minutes. Tubes were then centrifuged at 14,000 x g for 5 minutes to remove insoluble material, and the remaining whole cell extract was removed. Whole cell extract protein concentration was quantified using a total protein assay. 30 µg of the cell lysate was removed and assayed for human IL-33.

Condition	(pg/mL)
Untreated	23.7
Treated	25.6

Tissue Lysates - Human placenta was rinsed with PBS, cut into 1-2 mm pieces, and homogenized with a tissue homogenizer in PBS. An equal volume of RIPA buffer containing protease inhibitors was added and tissues were lysed on ice for 30 minutes with gentle agitation. Debris was then removed by centrifugation. A BCA was done on the supernate to determine total protein concentration. 15 µg of the tissue lysate was removed, assayed for human IL-33, and measured 102 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant human IL-33.

The factors listed below were prepared at 4000 pg/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 4000 pg/mL in a mid-range IL-33 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

IL-1α
IL-1β
IL-1ra
IL-1 RAcP
IL-1 RAPL1
IL-1 Rrp2
IL-18
IL-18 R
IL-18 Rβ
IL-36α
IL-36β2
IL-36γ
IL-36Ra
IL-37
IL-38
ST2/IL-1 R4

Recombinant mouse:

IL-1β
IL-33
IL-36α
IL-36β
IL-36γ
IL-36Ra
IL-18
ST2

Recombinant rat:

IL-1α
IL-1β
IL-1 Ra
IL-18

Recombinant equine:

IL-1β
IL-1Ra

Recombinant porcine:

IL-1α
IL-1β
IL-1Ra
IL-18

Recombinant cotton rat:

IL-1α
IL-1β

Recombinant rhesus macaque:

IL-1β
IL-18

Other recombinants:

canine IL-1β
guinea pig IL-1β
feline IL-18

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PLATE LAYOUT

Use this plate layout to record standards and samples assayed.