Quantikine® ELISA

Mouse/Rat IL-33 Immunoassay

Catalog Number M3300

For the quantitative determination of mouse or rat Interleukin 33 (IL-33) concentrations in cell culture supernates, 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

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

IL-33, also known as NF-HEV and DVS 27, is a 30 kDa pro-inflammatory protein with intracellular and extracellular activities (1, 2). IL-33 is constitutively expressed in smooth muscle and airway epithelia. It is upregulated by inflammatory stimulation in these cells, keratinocytes, and dermal fibroblasts and by mechanical strain in cardiac fibroblasts (3-5). Mouse IL-33 is synthesized as a 266 amino acid (aa) molecule that contains an N-terminal nuclear localization signal, a helix-turn-helix motif, and a C-terminal region with structural homology to IL-1 family cytokines. Full length IL-33 interacts with nuclear chromatin and functions as a transcriptional repressor (3, 4, 6). Cleavage of full length IL-33 by Caspase-1-like proteases leads to secretion of an 18 kDa C-terminal fragment that is known as mature IL-33 (7-9). Mature mouse IL-33 shares 57% and 90% aa sequence identity with human and rat IL-33, respectively. It shares less than 25% aa sequence identity with other IL-1 family proteins.

Mature IL-33 binds the transmembrane receptor ST2L/IL-1 R4 which subsequently associates with IL-1 R3 (also known as IL-1 RAcP) to enable IL-33 dependent activation of NFκB (7, 10-13). IL-1 R3 is a shared signaling subunit that also associates with IL-1 R1 and IL-1 R6. Alternate splicing of ST2L generates ST2, a soluble decoy receptor that is elevated in the serum of asthma and heart failure patients (5, 14). ST2 association with IL-33 blocks ST2L-dependent signaling and the immunologic and cardiac effects of IL-33 (5, 14).

Secreted IL-33 promotes Th2-biased immune responses, resulting in eosinophilia and allergic inflammation (15). In Th2 cells, it upregulates the production of IL-4, IL-5, and IL-13 as well as ST2L (7, 12). In mast cells, it enhances the production of several cytokines and chemokines but does not trigger degranulation (16 - 19). It inhibits mast cell apoptosis and functions as a chemoattractant for Th2 cells to sites of inflammation (17, 20). The IL-33/ST2L system has a protective role in the heart. IL-33 interactions with ST2L counteract the cardiac myocyte hypertrophy which is induced by angiotensin II or phenylephrine (5). In parallel with the induction of IL-33 in cardiac fibroblasts, ST2 is induced in cardiac myocytes by mechanical stress (5).

The Quantikine® Mouse/Rat IL-33 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure mouse or rat IL-33 in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant mouse mature IL-33 and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate both recombinant mouse and rat IL-33. Results obtained using natural IL-33 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 or rat IL-33.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A polyclonal antibody specific for mouse/rat IL-33 has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any IL-33 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse/rat IL-33 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-33 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 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.
- 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.

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 MSDS on our website prior to use.

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
Mouse/Rat IL-33 Microplate	893652	96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody specific for mouse/rat 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.*
Mouse/Rat IL-33 Conjugate	893653	12 mL of a polyclonal antibody specific for mouse/rat IL-33 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Mouse/Rat IL-33 Standard	893654	Recombinant mouse IL-33 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Mouse/Rat IL-33 Control	893655	Recombinant IL-33 in a buffered protein base with preservatives; lyophilized. The assay value of the control should be within the range specified on the label.	
Assay Diluent RD1-40	895513	12 mL of a buffered protein solution with preservatives.	
Calibrator Diluent RD5-17	895512	21 mL of a buffered protein solution with preservatives.	
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	895174	23 mL of diluted hydrochloric 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.
- Test tubes for dilution of standards.

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.

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.

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.*

REAGENT PREPARATION

Bring all reagents to room temperature before use.

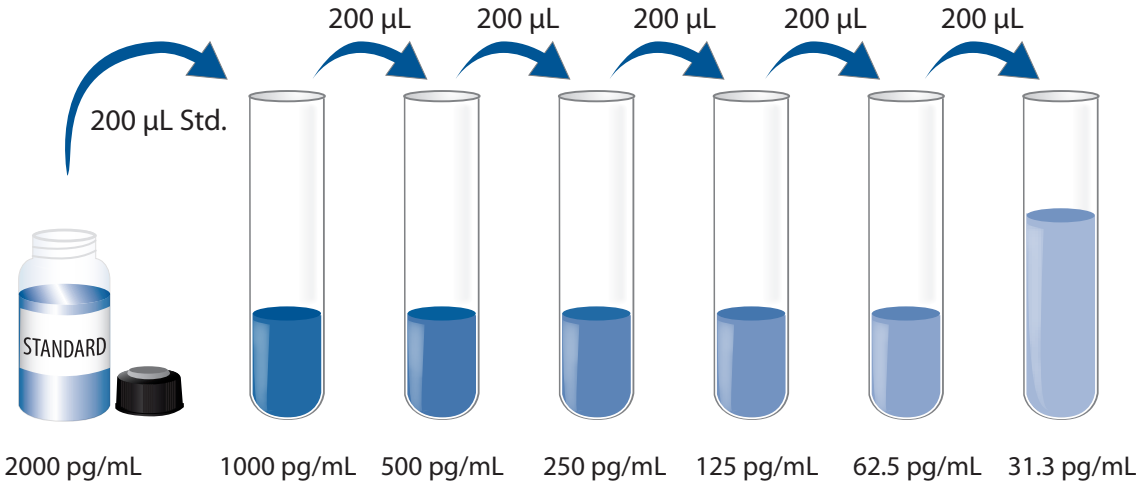
Mouse/Rat IL-33 Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

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 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 µL of the resultant mixture is required per well.

Mouse/Rat IL-33 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse/Rat IL-33 Standard with Calibrator Diluent RD5-17. Do not substitute other diluents. This reconstitution produces a stock solution of 2000 pg/mL. Allow the stock solution to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Pipette 200 µL of Calibrator Diluent RD5-17 into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Mouse/Rat IL-33 Standard (2000 pg/mL) serves as the high standard. Calibrator Diluent RD5-17 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 µL of Assay Diluent RD1-40 to each well.
4. Add 50 µL of standard, control, or sample per well. Mix gently by tapping the plate frame for 1 minute. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on the benchtop.
5. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. **Allow a 30 second soak time for each wash.** 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 µL of Mouse/Rat IL-33 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the benchtop.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature on the benchtop. **Protect from light.**
9. Add 100 µL 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

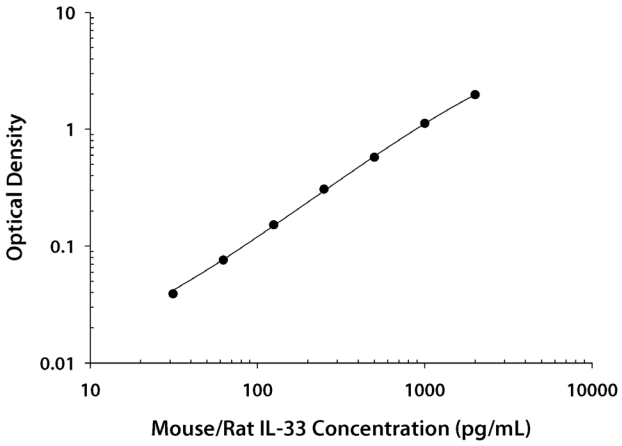
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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/rat 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	0.043 0.045	0.044	—
31.3	0.083 0.083	0.083	0.039
62.5	0.117 0.123	0.120	0.076
125	0.191 0.200	0.196	0.152
250	0.335 0.364	0.350	0.306
500	0.609 0.626	0.618	0.574
1000	1.163 1.169	1.166	1.122
2000	1.981 2.051	2.016	1.972

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 forty-one 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	41	41	41
Mean (pg/mL)	58.2	298	630	57.6	282	599
Standard deviation	5.6	17.4	33.5	5.89	16.3	30.0
CV (%)	9.6	5.8	5.3	10.2	5.8	5.0

RECOVERY

The recovery of mouse/rat IL-33 spiked to three levels throughout the range of the assay in various matrices was evaluated.

Mouse Samples	Average % Recovery	Range
Cell culture supernates (n=4)	104	91-116%
Serum (n=4)	90	81-103%
EDTA plasma (n=4)	102	96-113%
Heparin plasma (n=4)	99	83-111%

Rat Samples	Average % Recovery	Range
Cell culture supernates (n=4)	104	94-116%
Serum (n=4)	93	86-101%
EDTA plasma (n=4)	93	83-100%
Heparin plasma (n=4)	93	87-99%

SENSITIVITY

Forty seven assays were evaluated and the minimum detectable dose (MDD) of mouse/rat IL-33 ranged from 2.8-14.3 pg/mL. The mean MDD was 6.85 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 mouse mature IL-33 (aa 109-266) produced at R&D Systems®.

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of mouse/rat IL-33 in each matrix were diluted with calibrator diluent and assayed.

Mouse Samples		Cell culture supernates (n=4)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)
1:2	Average % of Expected	99	106	104	102
	Range (%)	96-102	104-109	99-109	98-107
1:4	Average % of Expected	100	109	111	104
	Range (%)	94-104	107-110	109-115	95-116
1:8	Average % of Expected	105	108	118	102
	Range (%)	98-112	105-111	114-119	97-113
1:16	Average % of Expected	106	99	114	100
	Range (%)	99-113	92-104	109-120	90-117

Rat Samples		Cell culture supernates (n=4)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)
1:2	Average % of Expected	104	109	102	103
	Range (%)	101-110	102-111	97-106	100-107
1:4	Average % of Expected	99	110	100	100
	Range (%)	89-106	102-118	94-106	91-108
1:8	Average % of Expected	101	110	103	99
	Range (%)	90-109	102-116	93-110	91-104
1:16	Average % of Expected	105	110	103	96
	Range (%)	102-107	99-120	89-112	92-102

SAMPLE VALUES

Serum/Plasma - Samples were evaluated for the presence of mouse and rat IL-33 in this assay.

Mouse Samples	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=20)	75.1	25	ND-217
EDTA plasma (n=20)	66.5	20	ND-103
Heparin plasma (n=20)	79.2	15	ND-127

Rat Samples	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=20)	44.8	20	ND-66.4
EDTA plasma (n=20)	34.5	5	ND-34.5
Heparin plasma (n=20)	55.0	20	ND-75.7

ND=Non-detectable

Cell Culture Supernates:

Lungs from mice or rats were chopped into 1-2 mm pieces and cultured in 100 mL RPMI supplemented with 10% fetal bovine serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate or stimulated with 1.0 µg/mL lipopolysaccharide (LPS) for 24 hours. Aliquots of the cell culture supernates were removed and assayed for levels of mouse/rat IL-33.

Lung Tissue	Observed Levels (pg/mL)
Mouse stimulated	1132
Rat stimulated	2297
Rat unstimulated	3364

Spleens from mice or rats were chopped into 1-2 mm pieces and cultured in 100 mL RPMI supplemented with 10% fetal bovine serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate or stimulated with 1.0 µg/mL LPS for 18 hours. Aliquots of the cell culture supernates were removed and assayed for levels of mouse/rat IL-33.

Spleen Tissue	Observed Levels (pg/mL)
Mouse stimulated	109
Mouse unstimulated	166
Rat stimulated	247
Rat unstimulated	228

Brains from two mice were chopped into 1-2 mm pieces and cultured in 100 mL RPMI supplemented with 10% fetal bovine serum and stimulated with 1.0 µg/mL LPS for 18 hours. An aliquot of the cell culture supernate was removed, assayed for mouse/rat IL-33, and measured 165 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant mouse and rat IL-33.

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/rat IL-33 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:

IL-1α
IL-1β
IL-1 R1
IL-1 R2
IL-1 R4/ST2
IL-1 R9
IL-1ra
IL-18
IL-33 (aa 1-108)
SIGIRR

Recombinant human:

IL-1α
IL-1β
IL-1F7b
IL-1 R4/ST2
IL-1ra
IL-18
IL-18 R
IL-33 (aa 1-111)

Recombinant human mature IL-33 (aa 112-270) shows 6% cross-reactivity in this assay.

REFERENCES

1. Gadina, M. and C.A. Jefferies (2007) Sci. STKE pe31.
2. Barksby, H.E. *et al.* (2007) Clin. Exp. Immunol. **149**:217.
3. Onda, H. *et al.* (1999) J. Cereb. Blood Flow Metab. **19**:1279.
4. Carriere, V. *et al.* (2007) Proc. Natl. Acad. Sci. USA **104**:282.
5. Sanada, S. *et al.* (2007) J. Clin. Invest. **117**:1538.
6. Baekkevold, E.S. *et al.* (2003) Am. J. Pathol. **163**:69.
7. Schmitz, J. *et al.* (2005) Immunity **23**:479.
8. Black, R.A. *et al.* (1989) J. Biol. Chem. **264**:5323.
9. Martinon, F. *et al.* (2007) Semin. Immunopathol. **29**:213.
10. Xu, D. *et al.* (1998) J. Exp. Med. **187**:787.
11. Lohning, M. *et al.* (1998) Proc. Natl. Acad. Sci. USA **95**:6930.
12. Chackerian, A.A. *et al.* (2007) J. Immunol. **179**:2551.
13. Ali, S. *et al.* (2007) Proc. Natl. Acad. Sci. USA **104**:18660.
14. Hayakawa, H. *et al.* (2007) J. Biol. Chem. **282**:26369.
15. Kato, A. and R.P. Schleimer (2007) Curr. Opin. Immunol. **19**:711.
16. Allakhverdi, Z. *et al.* (2007) J. Immunol. **179**:2051.
17. Likura, M. *et al.* (2007) Lab. Invest. **87**:971.
18. Ho, L.H. *et al.* (2007) J. Leukoc. Biol. **82**:1481.
19. Moulin, D. *et al.* (2007) Cytokine **40**:216.
20. Komai-Koma, M. *et al.* (2007) Eur. J. Immunol. **37**:2779.