IL-33 (human), ELISA kit

Catalog # **ADI-900-201**

96 Well Enzyme-linked Immunosorbent Kit
For use with cell lysates, culture supernatants, plasma, serum, and synovial fluid

Reagents require separate storage conditions.

Check our website for additional protocols, technical notes and FAQs.

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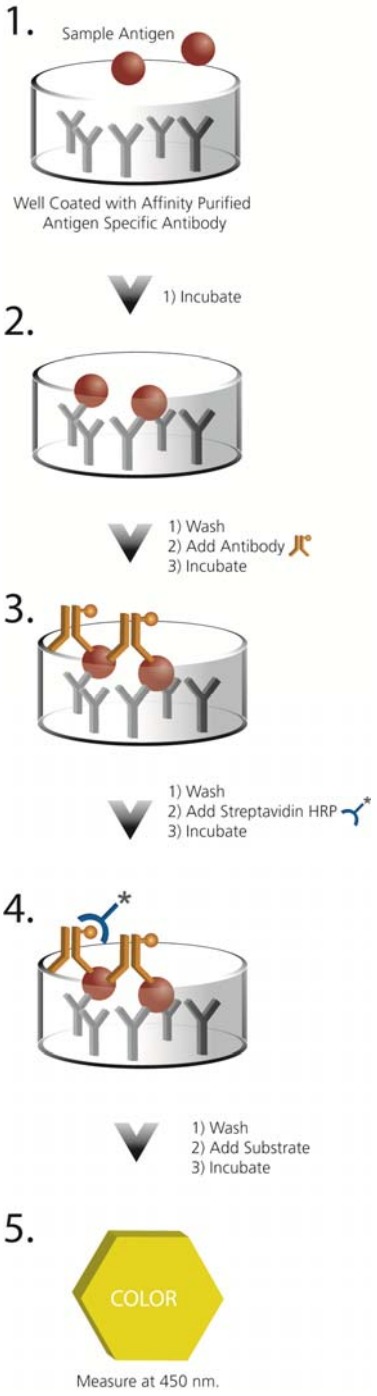


Description

The IL-33 (human) Enzyme-linked Immunosorbent Assay (ELISA) kit is a complete kit for the quantitative determination of IL-33 in cell lysates, culture supernatants, plasma, serum, and synovial fluid. Please read the complete kit insert before performing this assay.

Introduction

IL-33, also named Nuclear Factor from High Endothelial Venules (NF-HEV)[3], is a member of the Interleukin-1 (IL-1) family. Members of this family, such as IL-1α/β and IL-18, are known to play important roles in host defense, immune regulation, neuronal injury and inflammation[1, 2]. IL-33 has been reported to be the specific ligand for ST2[4], an orphan receptor of the IL-1 receptor family[5]. Stimulation of ST2 by IL-33 induces NF-κB and MAPK signaling pathways[4]. IL-33 is synthesized in response to cytokines such as IL-1β and TNF-α and plays an important role in TH2-associated immunology by triggering increased secretion of IL-5 and IL-13 from polarized TH2 cells[4]. IL-33 has also been found to be localized in the nucleus of human epithelial and endothelial cells[3], and can be present in serum and synovial fluid samples of patients with rheumatoid arthritis[6,8]. Overexpression of IL-33 in mice has been found to induce asthmatic symptoms, and enhance joint inflammation[7,8].

Principle

1. Samples and standards are added to wells coated with a monoclonal antibody to IL-33. The plate is then incubated.
2. The plate is washed, leaving only bound IL-33 on the plate. A yellow solution of biotinylated polyclonal antibody to IL-33 is then added. This binds to the IL-33 captured on the plate. The plate is then incubated.
3. The plate is washed to remove excess biotinylated antibody. A blue solution of streptavidin conjugated to horseradish peroxidase (HRP) is added to each well, binding to the biotinylated polyclonal IL-33 antibody.
4. The plate is washed to remove excess HRP conjugate. TMB Substrate solution is added. An HRP-catalyzed reaction generates a blue color in the solution.
5. Stop solution is added to stop the substrate reaction. The resulting yellow color is read at 450nm. The amount of signal is directly proportional to the level of IL-33 in the sample.

Materials Supplied

Do not mix components from different kit lots or use reagents beyond the expiration date of the kit.

The standard should be handled with care due to the known and unknown effects of the antigen.

Activity of conjugate is affected by nucleophiles such as azide, cyanide, and hydroxylamine.

Protect substrate from prolonged exposure to light.

Stop solution is caustic. Keep tightly capped.

1. **Assay Buffer 13**
   100 ml, Catalog No. 80-1627
   Tris buffered saline containing BSA and detergents

2. **IL-33 Standard**
   2 x 625 pg, Catalog No. 80-2353
   2 vials, each containing 625pg/vial of recombinant human IL-33

3. **IL-33 Clear Microtiter Plate**
   One plate of 96 wells, Catalog No. 80-2352
   A clear plate of break-apart strips coated with a monoclonal antibody specific for IL-33

4. **IL-33 ELISA(human), Antibody**
   10 ml, Catalog No. 80-2351
   A yellow solution of biotinylated polyclonal antibody to IL-33

5. **IL-33 Conjugate**
   10 ml, Catalog No. 80-2354
   A blue solution of streptavidin conjugated to horseradish peroxidase

6. **Wash Buffer Concentrate**
   27 ml, Catalog No. 80-1286
   Tris buffered saline containing detergents

7. **Extraction Reagent Concentrate**
   10 ml, Catalog No. 80-1502
   A solution of Tris and Igepal CA-630

8. **TMB Substrate**
   10 ml, Catalog No. 80-0350
   A solution of 3,3',5,5' tetramethylbenzidine (TMB) and hydrogen peroxide

9. **Stop Solution 2**
   10 ml, Catalog No. 80-0377
   A 1N solution of hydrochloric acid in water

10. **IL-33 Assay Layout Sheet**
    1 each, Catalog No. 30-0296

11. **Plate Sealer**
    3 each, Catalog No. 30-0012

Reagents require separate storage conditions.

Storage

All components of this kit, **except the Standard**, are stable at 4°C until the kit's expiration date. The standard must be stored at or below -20°C upon receipt. Shipping conditions may not reflect recommended storage temperatures.

Materials Needed but Not Supplied

1. Deionized or distilled water
2. Precision pipets for volumes between 5 µL and 1,000 µL
3. Repeater pipet for dispensing 100 µL
4. Disposable polypropylene beakers
5. Graduated cylinders
6. A microplate shaker
7. Lint-free paper for blotting
8. Microplate reader capable of reading 450 nm
9. Software for extrapolating sample values from optical density readings utilizing a four parameter logistic curve fit.
10. Software for extrapolating sample values from optical density readings utilizing a four parameter logistic curve fit, such as Assay Blaster! assay analysis software (Cat. #ADI-28-0002).

Reagent Preparation

Bring all reagents to room temperature for at least 30 minutes prior to opening.

Plastic tubes must be used for standard preparation.

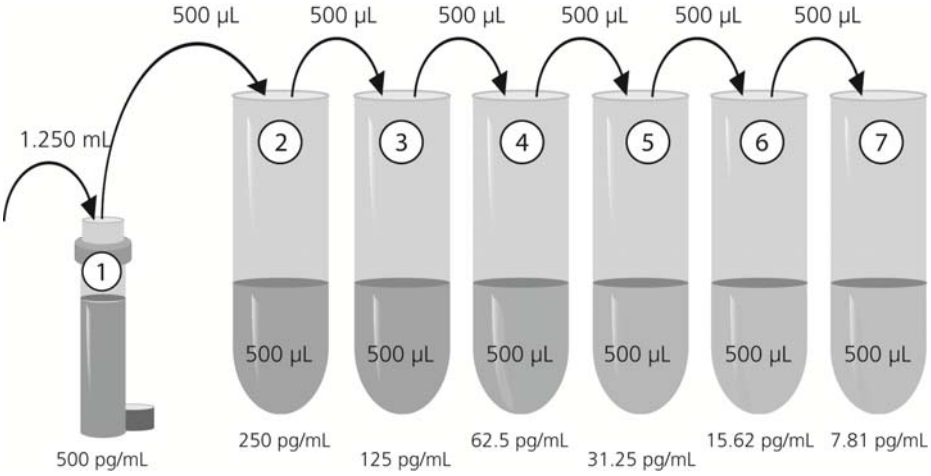
1. **Wash Buffer**

   Prepare the wash buffer by diluting 5ml of the supplied Wash Buffer Concentrate with 95ml of deionized water. This can be stored at room temperature until the kit's expiration date, or for 3 months, whichever comes first.

2. **Human IL-33 Standards**

   Allow the 625pg human IL-33 standard to warm to room temperature. Reconstitute one vial of Standard with 1.250 ml of the assay buffer for a 500 pg/ml stock vial. Vortex thoroughly, wait 5 minutes and vortex again prior to use.

   Label six disposable polypropylene 12x75 mm tubes #2 through #7. Pipet 500µl of the assay buffer into each tube. Remove 500 µl from reconstituted stock vial and add to tube #2 and vortex thoroughly. Remove 500 µl from tube #2 and add to tube #3. Vortex thoroughly. Continue this for tubes #3 through #7.



Diluted standards should be used within 1 hour of preparation. If standards are not to be used immediately, they should be placed on ice until use. The concentrations of the standards are labeled above.

3. **Extraction Reagent**

   Prepare the extraction reagent by diluting the supplied concentrate 1:5 with deionized water. This can be stored at room temperature until the kit's expiration date, or for 3 months, whichever comes first.

If buffers other than those provided are used in the assay, the end-user must determine the appropriate dilution and assay validation.

Samples must be stored frozen at or below -20° to avoid loss of bioactive analyte. Repeated freeze/thaw cycles should be avoided.

Sample Handling

The IL-33 (human), ELISA Kit is compatible with IL-33 samples in culture supernates, cell lysates, plasma, serum, and synovial fluid of human origin. Samples diluted sufficiently into the assay buffer can be read directly from a standard curve. Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens. An optional protocol is provided for clearing rheumatoid factor.

The minimum recommended dilutions to remove matrix interference in the assay are: 1:2 for synovial fluid, 1:4 for cell lysates, 1:8 for serum and plasma, and culture supernatants (with 10% serum supplement) may be assayed neat. Due to differences in cell types, number of cells, or total cellular protein concentration, lysates and supernatants may require a greater dilution to remove interference or to be read within the standard range. The optimal dilution for any experiment should be determined by the investigator.

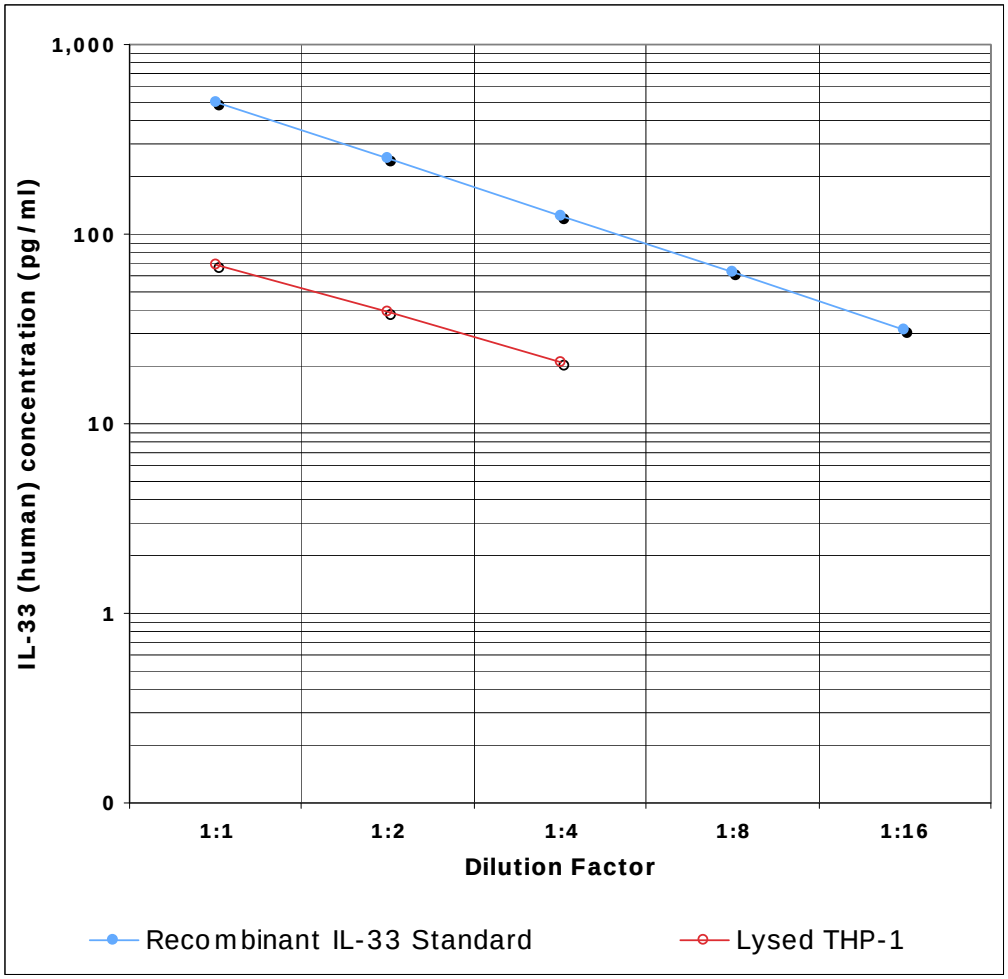
Dilutional Linearity

The minimum required dilution for several common samples was determined by serially diluting samples spiked with recombinant human IL-33 into the assay buffer and identifying the dilution at which linearity was observed. The assay buffer was spiked to the same concentration and used as a control to determine linearity.

Dilution Factor	Cell Supernatant ($1x10^6$ cells/ml)	Heparin Plasma (Pooled)	Serum (Pooled)	Synovial Fluid
Neat	92.4	23.2	31.3	
1:2	100.3	60.8	73.6	95.0
1:4	98.9	80.2	94.6	89.4
1:8	100.2	90.5	114.8	104.1
1:16	101.1	96.0	123.3	99.5

Parallelism

Parallelism experiments were carried out to determine if the recombinant human IL-33 standard accurately determines IL-33 concentrations in biological matrices. THP-1 (human monocytic leukemia cells) stimulated with lipopolysaccharide (LPS) and phorbol myristate acetate (PMA) were used to assess parallelism. Values were obtained using the cell lysates from treated cultures serially diluted in the assay buffer and assessed from a standard curve using four parameter logistic curve fitting. The observed values were plotted against the dilution factors. Parallelism of the curves demonstrates that the antigen binding characteristics are similar enough to allow the accurate determination of native analyte levels in diluted samples.



Spike and Recovery

After diluting each sample matrix to its minimum recommended dilution, recombinant human IL-33 was spiked at high, medium, and low concentrations. The recovery of the standard in spiked samples was compared to the recovery of identical spikes in the assay buffer. The mean and range of percent recovery at the three concentrations are indicated below for each matrix.

Sample Matrix	Minimum Recommended Dilution	Spike Concentration (pg/ml)	Recovery of Spike
Serum	1:4	400	82.9%
		100	81.8%
		25	70.2%
Heparin Plasma	1:8	400	92.3%
		100	93.9%
		25	89.5%
Culture Media + 10% FBS	1:2	400	99.0%
		100	97.5%
		25	89.9%
Synovial Fluid	1:16	400	73.8%
		100	71.9%
		25	71.2%

Samples must be stored frozen at or below -20° to avoid loss of bioactive analyte. Repeated freeze/thaw cycles should be avoided.

Cell Lysis Protocol

1. Incubate THP-1 cells in RPME supplemented with 10% FBS, L-glutamine and pen/strp/ampho for 48 hours.
2. Add Lipopolysaccharide (LPS) at 5 ug/mL and Phorbol 12-Myristate 13-acetate (PMA) at 50 nM. Incubate for 24 hours.
3. Wash to remove LPS and PMA. Add Sodium Azide at 1M. Incubate for 1 hour to induce necrosis.
4. Centrifuge at 1700 rpm for 10 minutes at room temperature to pellet any cells or cellular debris. Dilute Extraction Reagent 1:5 with deionized water. Add Extraction Reagent to lyse cells. Mix by inverting 10 minutes. Centrifuge to pellet cellular debris.
5. Collect supernatant in a clean tube.
6. The supernatant may be divided into aliquots and stored at or below -20C, or used immediately in the assay.

Samples must be stored frozen at or below -20° to avoid loss of bioactive analyte. Repeated freeze/thaw cycles should be avoided.

Plasma Preparation

1. Collect whole blood in an appropriate tube.
2. Centrifuge at 1000 x g for 15 minutes at 4˚C.
3. Place supernatant in a clean tube.
4. The supernatant may be divided into aliquots and stored at or below -20˚C, or used immediately in the assay.
5. Avoid repeated freeze-thaw cycles.

Serum Preparation

1. Collect whole blood in an appropriate tube containing anticoagulant (heparin or EDTA).
2. Centrifuge at 1300 x g for 15 minutes at room temperature.
3. Place top layer in a clean tube.
4. The supernatant may be divided into aliquots and stored at or below -20˚C, or used immediately in the assay.
5. Avoid repeated freeze-thaw cycles.

Optional Rheumatoid Factor (RF) Clearing Protocol

It is possible that RF present in samples can interfere with this assay[8]. To reduce potential interference by RF, samples can be pre-cleared by treating with 10% (v/v) protein A-sepharose beads overnight at 4°C[10].

Assay Procedure

Bring all reagents to room temperature for at least 30 minutes prior to opening.

Pre-rinse each pipet tip with reagent. Use fresh pipet tips for each sample, standard, and reagent.

All standards and samples should be run in duplicate.

Pipet the reagents to the sides of the wells to avoid possible contamination.

Prior to the addition of antibody, conjugate, and substrate, ensure there is no residual wash buffer in the wells. Remaining wash buffer may cause variation in assay results.

Refer to the Assay Layout Sheet to determine the number of wells to be used and return the remaining wells, with the desiccant, back to the mylar bag and seal. **Store unused wells at 4°C**.

1. Pipet 100 µl of the assay buffer into the S0 (0 pg/ml standard) wells.
2. Pipet 100 µl of Standards #1 through #7 into the appropriate wells.
3. Pipet 100 µl of the samples into the appropriate wells.
4. Seal the plate. Incubate at room temperature on a plate shaker for 1 hour at ~500 rpm.
5. Empty the contents of the wells and wash by adding 400 µl of wash solution to every well. Repeat the wash 3 more times for a total of **4 washes**. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
6. Pipet 100 µl of yellow biotinylated antibody into each well, except the Blank.
7. Seal the plate. Incubate at room temperature on a plate shaker for 1 hour at ~500 rpm.
8. Wash as above (Step 5).
9. Add 100 µl of blue conjugate to each well, except the Blank.
10. Seal the plate. Incubate at room temperature on a plate shaker for 30 minutes at ~500 rpm.
11. Wash as above (Step 5). Pipet 100 µl of Substrate Solution into each well.
12. Seal the plate. Incubate at room temperature on a plate shaker for 30 minutes at ~500 rpm.
13. Pipet 100 µl Stop Solution to each well.
14. Zero the plate reader against the Blank wells, read the optical density at 450 nm, preferably with correction between 570 and 590 nm. If the plate reader is not able to be blanked against the Blank wells, manually subtract the mean optical density of the Blank wells from all the readings.

Calculation of Results

Make sure to multiply sample concentrations by the dilution factor used during sample preparation.

Several options are available for the calculation of the concentration of IL-33 in the samples. We recommend that the data be handled by an immunoassay software package utilizing a four parameter logistic curve fitting program. Such software is often supplied by plate reader manufacturers. If data reduction software is not readily available, the concentration of IL-33 can be calculated as follows.

1. Calculate the average Net OD for each standard and sample by subtracting the average blank OD from the average OD for each standard and sample.

   Average Net OD = Average OD – Average Blanks OD

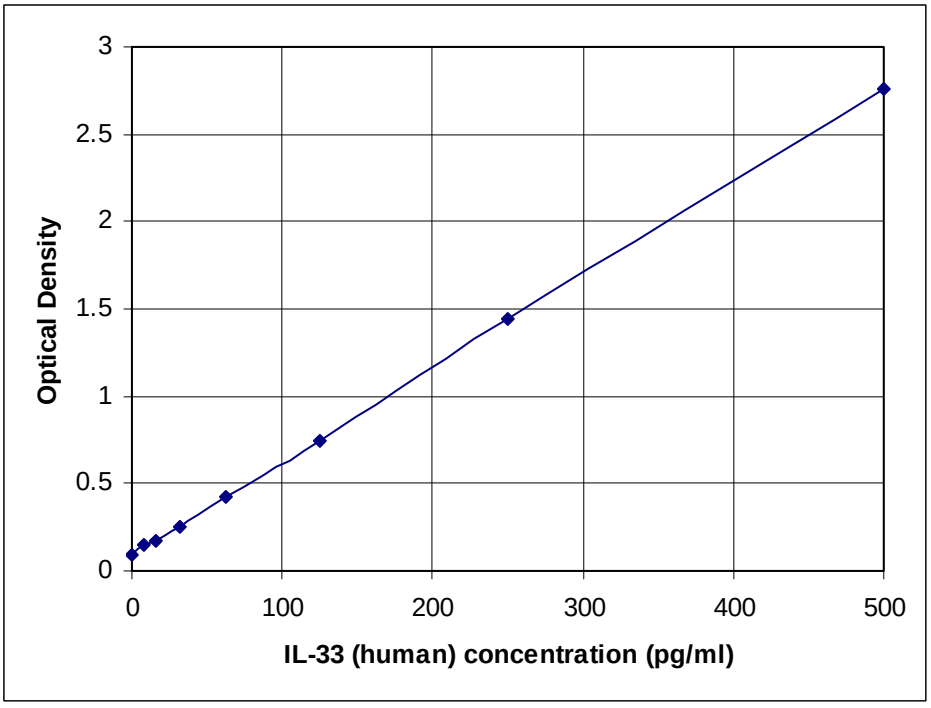
2. Plot the average Net OD for each standard versus IL-33 concentration in each standard. Approximate a straight line through the points. The concentration of the unknowns can be determined by interpolation.

Samples with concentrations outside of the standard curve range will need to be reanalyzed using a different dilution.

Typical Results

The results shown below are for illustration only and should not be used to calculate results from another assay.

Sample	Net OD	IL-33(pg/ml)
S0	0.099	0
S1	2.825	500
S2	1.473	250
S3	0.770	125
S4	0.442	62.5
S5	0.268	31.25
S6	0.184	15.62
S7	0.145	7.81
Unknown #1	1.851	413.4
Unknown #2	0.473	109.4



Performance Characteristics

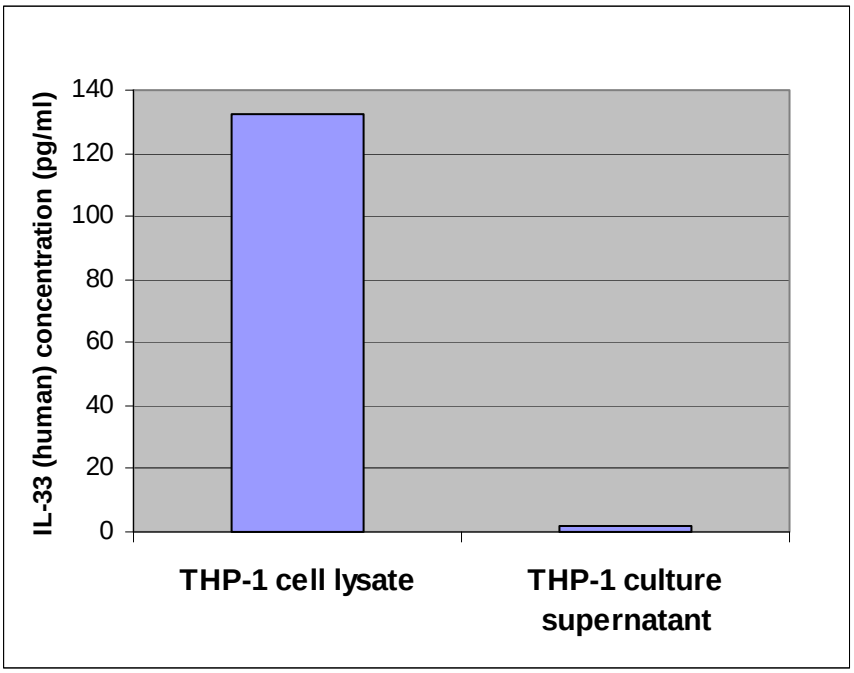
Specificity

Cross reactivity was assessed with IL-1α, IL-2, IL-10, MCP-1, IL-13, MIP-1β, IL-4, IL-6, IL-8, TNF-α, IFN-γ, IL-1β, IL-5, IL-15, IL-17, IL-21, IL-29 and RANTES by diluting them in the assay buffer at several concentrations. Results show no cross reactivity.

Mouse IL-33 recombinant shows less than 2% cross reactivity in this assay. A sample containing 25 ng/ml of mouse IL-33 read 400 pg/ml.

Stimulation Experiment

THP-1 cells were treated with LPS and PMA as described in Luthi *et al*[9]. Cell lysate and supernatant are shown.



Sensitivity

The sensitivity or limit of detection of the assay is 1.7pg/ml. The sensitivity was determined by interpolation at 2 standard deviations above the mean signal at background (0pg/ml) using data obtained from 20 low and 20 zero standards.

Precision

Intra-assay precision was determined by assaying 20 replicates of three buffer controls containing recombinant human IL-33 in a single assay.

pg/ml	%CV
400	2.8
100	1.9
25	1.8

Inter-assay precision was determined by measuring buffer controls of varying recombinant human IL-33 concentrations in multiple assays over several days.

pg/ml	%CV
250	2.4
62.5	4.4
7.8	4.0

References

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Notes

Use of Product

This product contains research chemicals. As such, they should be used and handled only by or under the supervision of technically qualified individuals. This product is not intended for diagnostic or human use.

Warranty

Enzo Life Sciences International, Inc. makes no warranty of any kind, expressed or implied, which extends beyond the description of the product in this brochure, except that the material will meet our specifications at the time of delivery. Enzo Life Sciences International, Inc. makes no guarantee of results and assumes no liability for injuries, damages or penalties resulting from product use, since the conditions of handling and use are beyond our control.

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