ImmunoDiagnostics Limited

FOR RESEARCH USE ONLY

Mouse IL-33 ELISA Kit

(Catalog Number: 32750)

For the quantitative determination of IL-33 in mouse serum, plasma or cell culture supernate samples

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Version 2.0

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PRINCIPLE OF THE ASSAY

This assay is a quantitative sandwich enzyme-linked immunosorbent assay (ELISA). The microtiter plate is pre-coated with a polyclonal antibody specific for mouse IL-33. Standards and samples are pipetted into the wells and any mouse IL-33 present is bound by the immobilized antibody. After washing away any unbound substances, a biotin labelled polyclonal antibody specific for mouse IL-33 is added to the wells. After wash step to remove any unbound reagents, streptavidin-horseradish peroxidase conjugate (STP-HRP) is added. After the last wash step, an HRP substrate solution is added and color develops in proportion to the amount of mouse IL33 bound initially. The assay is stopped, and the optical density of the wells is determined using a microplate reader. Since the increases in absorbance are directly proportional to the amount of captured mouse IL-33, the unknown sample concentration can be interpolated from a reference curve included in each assay.

INTENDED USE

This Mouse IL-33 ELISA kit is designed for quantification of mouse IL-33 in serum, plasma samples and cell culture supernatant samples.

REAGENTS SUPPLIED

Each kit is sufficient for one 96-well plate and contains the following components:

1. Microtiter Strips (96 wells), coated with a polyclonal antibody against mouse IL-33, sealed
2. 10×Wash buffer, 50 mL
3. 5×Assay buffer, 20 mL
4. 100×Detection antibody solution, a biotin labelled polyclonal antibody against mouse IL-33, 0.12 mL
5. Mouse IL-33 standard, 2000 pg of recombinant mouse IL-33, lyophilized
6. 200×STP-HRP solution, 0.06 mL
7. Substrate solution, 12 mL, ready for use
8. Stop solution, 12 mL, ready for use

OTHER MATERIALS REQUIRED, BUT NOT PROVIDED

1. Pipettes and pipette tips
2. 96-well plate or manual strip washer
3. Buffer and reagent reservoirs
4. Paper towels or absorbent paper
5. Plate reader capable of reading absorbency at 450 nm
6. Distilled water or deionized water

STORAGE

The kit should be stored at 2-8°C upon receipt, and all reagents should be equilibrated to room temperature before use. Remove any unused antibody-coated strips from the mouse IL-33 microtiter plate, return them to the foil pouch and re-seal. Once opened, the strips may be stored at 2-8°C for up to one month.

PREPARATION OF REAGENTS

Bring all reagents and materials to room temperature before assay.

A. 1×Assay buffer

Prepare 1×Assay buffer by mixing the 5×Assay buffer (20 mL) with 80 mL of distilled water or deionized water. If precipitates are observed in the 5×Assay buffer bottle, warm the bottle in a 37°C water bath until the precipitates disappear. The 1×Assay buffer may be stored at 2-8°C for up to one month.

B. 1×Wash buffer

Prepare 1×Wash buffer by mixing the 10×Wash buffer (50 mL) with 450 mL of distilled water or deionized water. If precipitates are observed in the 10×Wash buffer bottle, warm the bottle in a 37°C water bath until the precipitates disappear. The 1×Wash buffer may be stored at 2-8°C for up to one month.

C. 1×Detection antibody solution

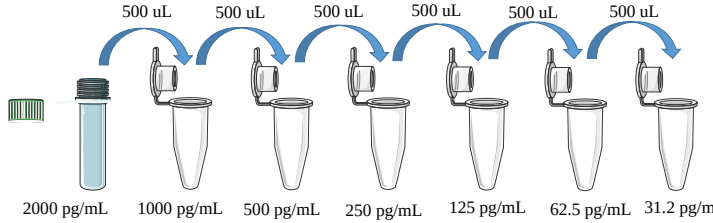
Spin down the 100×Detection antibody solution briefly and dilute the desired amount of the antibody 1:100 with 1×Assay buffer, 100 µL of the 1×Detection antibody solution is required per well. Prepare only as much 1×Detection antibody solution as needed. Return the 100×Detection antibody solution to 2-8°C immediately after the necessary volume is removed.

D. 1×STP-HRP solution

Spin down the 200×STP-HRP solution briefly and dilute the desired amount of the 200×STP-HRP solution 1:200 with 1×Assay buffer, 100 µL of the 1×STP-HRP solution is required per well. Prepare only as much 1×STP-HRP solution as needed. Return the 200×STP-HRP solution to 2-8°C immediately after the necessary volume is removed.

PREPARATION OF STANDARDS AND SAMPLES

Mouse IL-33 Standards: Reconstitute the lyophilized standard with 1 mL of 1×Assay buffer to generate a standard stock solution of 2000 pg/mL. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare serially diluted standards using 1×Assay buffer as shown below.



1×Assay buffer serves as the zero standard (0 pg/mL). The reconstituted standard stock should be aliquoted and stored at -20ºC for one month. Avoid repeating freezing/thawing cycles. Please do not store the diluted standard solutions.

Sample Preparation:
Serum, plasma or cell culture supernate sample generally requires appropriate dilution in the 1×Assay buffer. It is recommended that the users establish their own dilution factors based on the concentration range of their samples.

ASSAY PROCEDURE

It is recommended that all standards and samples be assayed in duplicate.

1. Add 100 µL of standard or sample per well, incubate at room temperature for 2 hours.
2. Discard the content and tap the plate on a clean paper towel to remove residual solution in each well. Add 300 µL of 1×Wash buffer to each well and incubate for 1 minute. Discard the 1×Wash buffer and tap the plate on a clean paper towel to remove residual wash buffer. Repeat the wash step for a total 3 washes.
3. Add 100 µL of 1×Detection antibody solution to each well, incubate at room temperature for 1 hour.
4. Wash each well 3 times as in step 2.
5. Add 100 µL of 1×STP-HRP solution to each well, incubate at room temperature for 20 minutes.
6. Wash each well 4 times as described in step 2.
7. Add 100 µL of Substrate solution to each well, incubate at room temperature for 15 minutes. **Protect from light.**
8. Add 100 µL of Stop solution to each well, gently tap the plate frame for a few seconds to ensure thorough mixing.
9. Measure absorbance of each well at 450 nm immediately.

CALCULATION

1. Subtract the absorbance of the blank from that of standards and samples.
2. Generate a standard curve by plotting the absorbance obtained (y-axis) against mouse IL-33 concentrations (x-axis). The best fit line can be generated with any curvefitting software by regression analysis. Any curve of 4-parameter or log-log curve fitting can be used for calculation.
3. Determine mouse IL-33 concentration of samples from standard curve and multiply the value by the dilution factor.

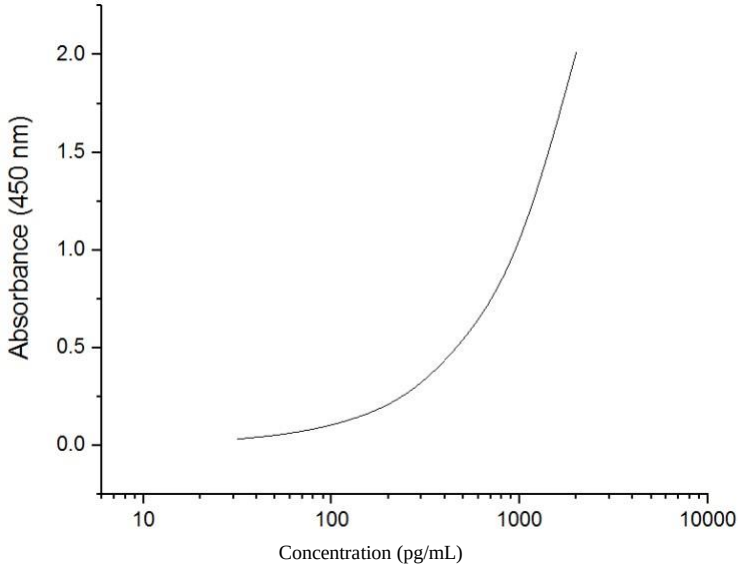
TYPICAL STANDARD CURVE

The following standard curve is provided for demonstration only. A standard curve should be generated for each set of sample assay.

Mouse IL-33 (pg/mL)	Absorbance (450 nm)	Blanked Absorbance
0	0.11	0
31.25	0.145	0.027

62.5	0.179	0.062
125	0.236	0.118
250	0.319	0.202
500	0.65	0.532
1000	1.2	1.083
2000	2.35	2.233

Mouse IL-33 standard curve (4 parameters)



ASSAY CHARACTERISTICS

A. Sensitivity

The lowest level of mouse IL-33 that can be detected by this assay is 6.5 pg/mL.

B. Precision

Intra-Assay Precision (Precision within an assay)
Three samples of known concentration were tested 8 times on one plate.

Sample	Mean (pg/mL)	SD (pg/mL)	CV (%)
1	1387.6	56.4	4.1

2	700.1	20.7	6.5
3	56.4	3.0	5.7

Intra-Assay Precision (Precision within an assay)
Three samples of known concentration were tested 8 times on separate plates.

Sample	Mean (pg/mL)	SD (pg/mL)	CV (%)
1	1425.7	33.5	2.4
2	713.8	29.6	4.2
3	146.3	18.3	12.5

C. Spiking

Cell culture supernate samples were assayed by adding 90 µL of sample and 10 µL of spike stock solution calculated to yield the intended 0, 150, 750 or 1500 pg/mL spike concentration.

Sample	**Spiked level**	**Expected (pg/mL)**	**Observed (pg/mL)**	**Recovery %**
Pooled cell culture medium (4X)	Low spike (150 pg/mL)	96.4	111.4	115.6
	Medium spike (750 pg/mL)	696.4	719.4	103.3
	High spike (1500 pg/mL)	1319.4	1343.4	101.8

D. Linearity

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of mouse IL-33 were serially diluted with the 1×Assay buffer to produce samples with values within the dynamic range of the assay.

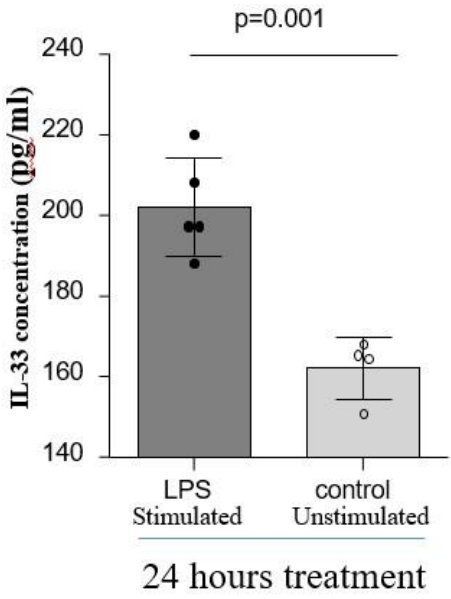
Serial dilution	Measured (pg/mL)	Expected (pg/mL)	Recovery %
Neat	1427.7	1427.7	100
1:2	703.7	713.9	98.6
1:4	329.7	356.9	92.4
1:8	171.2	178.5	95.9

F. Validation

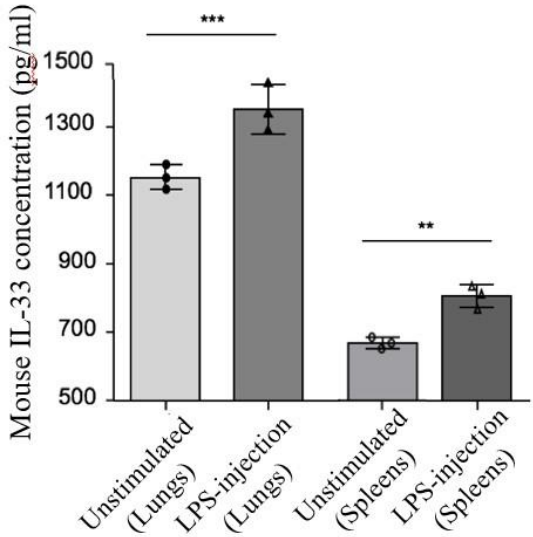
Cell culture supernates:

Lungs from mice were chopped into 1-2 mm pieces and cultured in 15 mL RPMI supplemented with 10% FBS, 50 μM β-ME, 100 U/mL penicillin, and 100 μg/mL streptomycin sulfate or stimulated with 1.0 μg/mL lipopolysaccharide (LPS) for 24 hours. Cell culture medium was removed and assayed for levels of mouse IL-33. In addition, we also collected the cell lysates and measured IL-33 levels and compared with the total protein levels in the cell lysates.

Sample (cell lysates)	IL-33 levels/ total protein (pg/mg)
LPS-stimulated	381.7
Unstimulated	190.9



A per-body-weight dose (100 ug/10g) LPS were injected into mice (i.p.) and after 12 hours mice were sacrificed. Lungs and spleens from mice were chopped into 1-2 mm pieces and cultured in 6-well plate. After 24 hours incubation, cell culture medium was removed and assayed for levels of mouse IL-33.



SUMMARY OF ASSAY PROCEDURE

Add 100 µL of standard or sample to each well.
↓
Incubate at room temperature for 2 hours.
↓
Aspirate and wash each well three times.
↓
Add 100 µL of 1×Detection antibody solution to each well.
↓
Incubate at room temperature for 1 hour.
↓
Aspirate and wash each well three times.
↓
Add 100 µL of 1×STP-HRP solution to each well.
↓
Incubate at room temperature for 20 minutes.
↓
Aspirate and wash each well four times.
↓
Add 100 µL of Substrate solution to each well.
↓
Incubate at room temperature for 15 minutes.
↓
Add 100 µL of Stop solution to each well.
↓
Measure absorbance of each well at 450 nm.
↓
Calculation