Human IL-33 ActivELISA™

For the Detection of Human IL-33 in the Supernatant

Catalog No.: IMK-542XL-2
Version 2011

2 x 96 well format (192 tests)

Expiration is 6 months from date of receipt of Kit.

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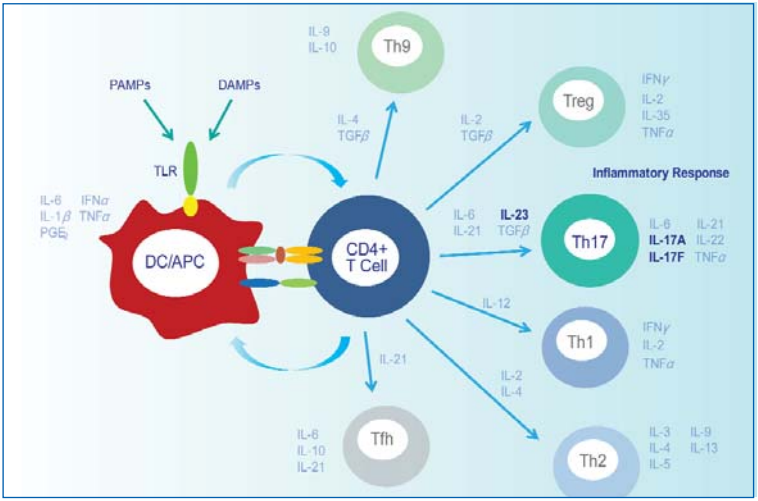
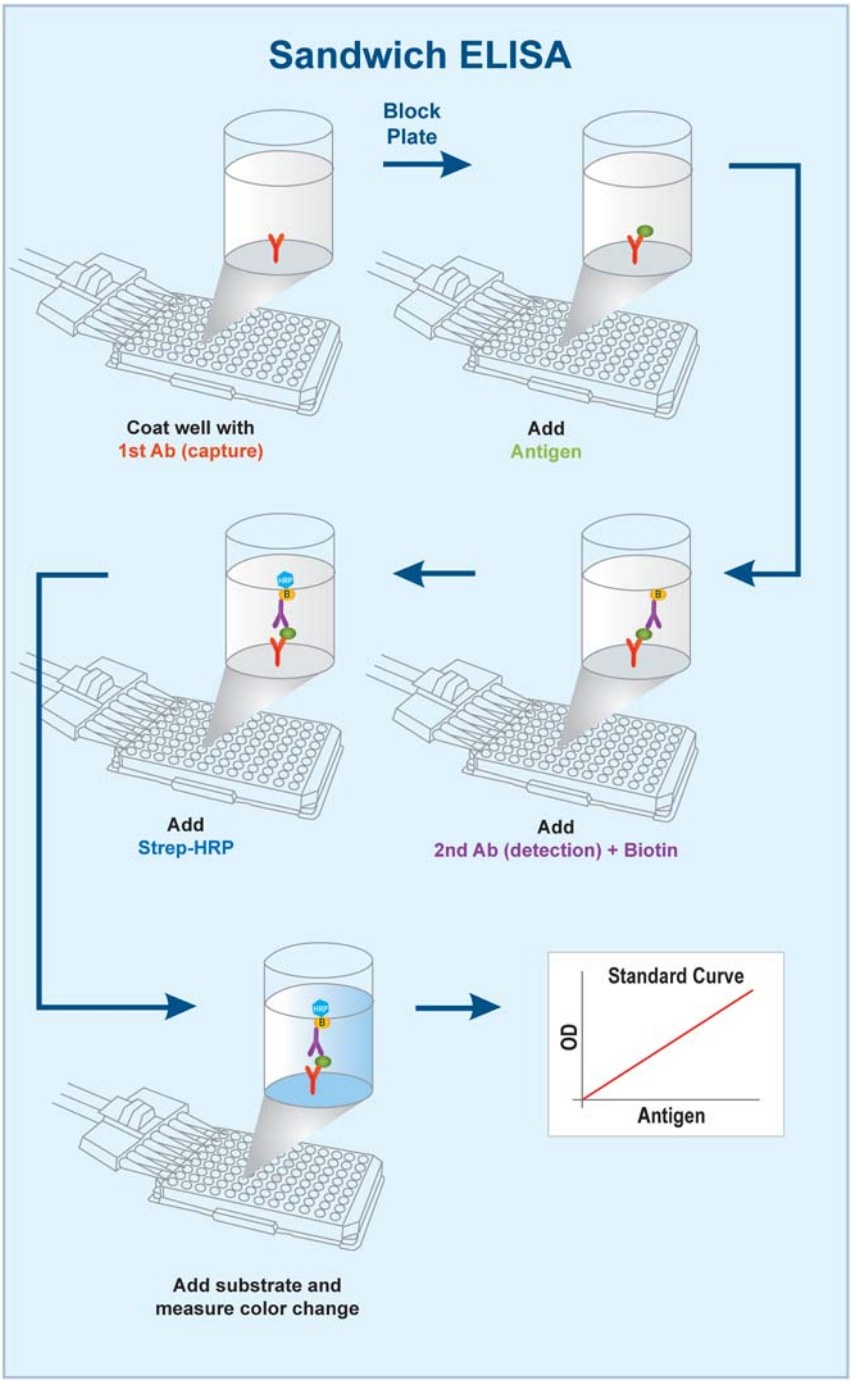
I. BACKGROUND

IL-33 is a member of the Interleukin-1 family. It performs dual roles, both as a proinflammatory cytokine and an intracellular nuclear factor with transcriptional regulatory properties, as well. It is synthesized as a 30-kDa precursor, which is cleaved by Caspase 1 to generate an 18-kDa fragment, which is sufficient to activate signaling by the IL-33 receptor T1/ST2. IL-33 induces helper T cells, mast cells, eosinophils and basophils to produce type 2 cytokines. It directly stimulates eosinophil differentiation from CD117+ progenitors in an IL-5-dependent manner. IL-33/ST2 signaling pathway activates airway eosinophils that exacerbate airway inflammation in an autocrine and paracrine manner. IL-33 signaling through ST2 receptor involves the recruitment of the adaptor protein MyD88, IRAK1, IRAK4, and TRAF6, activating MAPKs and NF-kappaB, thereby regulating the transcription of cytokines typically associated with a Th2 response such as IL-4, IL-5, and IL-13 (Foo Y et al, 2011). The affinity of IL-33 for ST2L is enhanced in the presence of IL-1RAcP. IL-33 plays a significant role in the amplification of AAM polarization and chemokine production, which contribute to innate and Ag-induced airway inflammation. It strongly amplifies the expression of Arginase I and Ym1 as well as the production of CCL24 and CCL17, which may further recruit inflammatory cells. It also enhanced the eosinophil-mediated differentiation of airway macrophages toward the alternatively activated macrophage phenotype in an IL-13-dependent manner. IL-33 induces TLR4 expression on macrophages, rendering them more responsive to LPS stimulation. Unprocessed IL-33 accumulates in the nucleus, where it acts as a transcriptional repressor. IL-33 orchestrates its potential role as a mediator in the pathophysiology of many diseases such as cardiovascular disorder, asthma and rheumatoid arthritis (Bartosz Stolarski et al, 2011).





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II. OVERVIEW

The human IL-33 ActivELISA™ Kit measures free IL-33 in the supernatant of activated cells. Standard protocols for detecting IL-33 activity include ELISA and Western Blot. The IL-33 ActivELISA™ can be completed in one day using a sandwich ELISA protocol. The anti-IL-33 antibody is coated on a plate which then captures free IL-33. The amount of bound IL-33 is detected by adding a second biotinylated anti-IL-33 antibody followed by HRP-conjugated streptavidin. ABTS substrate is then added and the concentration is determined by colorimetric detection in an ELISA plate reader.

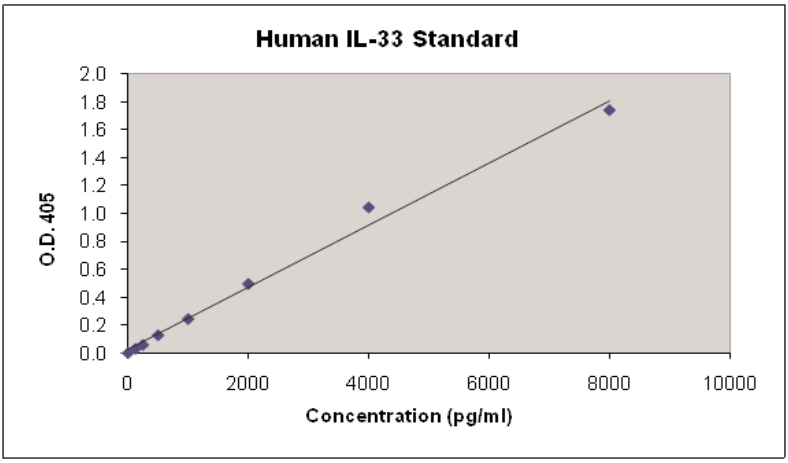
III. ADVANTAGES

- Multiple samples can be analyzed in a low-volume, high-throughput format
- Full analysis complete in just hours
- High sensitivity: Detects human IL-33 at concentrations as low as 15 pg/ml.

IV. EXPERIMENT

EXAMPLE STANDARD

Following the provided protocol, IL-33 standard was titrated to determine detectible levels.



V. KIT COMPONENTS AND STORAGE

Kit Components and storage for the IMK-542XL-2 IL-33 ActivELISA™ Module.

REAGENTS (4°C STORAGE)		
KC-542-1	**100X Capture Antibody***	250 µl
KC-542-2	**100X Detection Antibody***	250 µl
KC-100	**BSA**	4 x 0.5 g
KC-101	**20X Wash Buffer**	50 ml
KC-145	**Coating Buffer**	25 ml
KC-142	**10X Blocking Buffer**	20 ml
KC-143	**HRP-Conjugated Streptavidin**	10 µl
KC-144	**ABTS Substrate**	2 x 12 ml
REAGENTS (-20°C, STORAGE--NON FROST-FREE FREEZER)		
KC-542-3	**Recombinant IL-33 Standard**	1 vial, lyophilized (0.1 µg)
ADDITIONAL ITEMS INCLUDED		
ELISA Strip Well Plates		2
IMK-542XL-2 Manual		1

** Contains 0.02 % Sodium azide. Sodium azide is highly toxic.*

Additional items required for the ELISA (not included in the IMK-542XL-2 IL-33 ActivELISA™ Module):

- 96-well ELISA plate reader

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VI. PREPARATION OF REAGENTS: IL-33 ActivELISA™

NOTE: *The included buffers and reagents are optimized for use with this kit. Substitution with other reagents is not recommended and may not give optimal results.*

1X Wash Buffer: Prepare 1X Wash Buffer by diluting 20X Wash Buffer (KC-101) in distilled water. The diluted Wash Buffer may be stored at 4°C, however we recommend preparing fresh 1X Wash Buffer for each experiment.

1X Blocking Buffer: Prepare 1X Blocking Buffer fresh prior to experiment. Dilute 10X Blocking Buffer (KC-142) with distilled water. Dissolve 0.5 g BSA (KC-100) in 50 ml of 1X Blocking Buffer in a sterile bottle.

VII. IL-33 ActivELISA™ PROTOCOL

This kit allows for the quantitative measurement of IL-33 in a 96-well micro titer format. All 96-wells may be used at one time or you may only use the wells as required by your experimental design. Use of duplicate wells for each time point is recommended to obtain accurate results.

APPROPRIATE CONTROLS TO INCLUDE

Following is a list of suggested controls to include with each analysis:

1. No capture antibody added to well
2. No supernatant added to well
3. No capture antibody or supernatant added to well
4. Positive control: use a sample/supernatant known to contain human IL-33 or a recombinantly expressed human IL-33
5. Negative control: fresh cell culture media

ELISA PROTOCOL

Note: *This protocol is designed for a full 96-well assay. You can prepare lower volumes of reagents if you are not using the entire plate for one test.*

1. **Coating:** Dilute 120 µl of 100X Capture Antibody (KC-542-1) in 12 ml Coating Buffer (KC-145). Pipet 100 µl of 1X Capture Antibody into each well (A1 through H1 and A2 through H2 for the standard and any of columns 3 through 12 for your samples), seal the plate and incubate overnight (12-24 h) at room temperature. Wash the coated wells twice with 300 µl of 1X Wash Buffer.

2. **Blocking:** Add 200 µl of prepared 1X Blocking Buffer to each well to block the remaining reactive surface. Seal the plate and incubate for 30 min to 1 h at RT on a shaker set to 100 RPM.

3. **Prepare IL-33 Standard:** Quick spin down the Recombinant IL-33 Standard vial (KC-542-3) and add 100 µl of sterile deionized H20. Vortex gently to dissolve. Allow the vial to sit for 15 min, then vortex again. Stock standard concentration is 1 ug/ml. The stock standard may be stored in 4°C for up to 60 days after reconstitution. Suggested dilution of stock standard: add 4 ul of stock standard (1 ug/ml) in to 996 ul of 1X blocking buffer, this makes 4000 pg/ml*. Set up a standard curve following the directions below:

   - Remove Blocking Buffer from wells by flicking into an appropriate waste container and gently tapping the plate face-down on paper towels. Replace with 100 µl of fresh prepared 1X Blocking Buffer in each well **B1** through **H1** and **B2** through **H2** for the standard.
   - Pipette 200 µl stock Recombinant IL-33 Standard (4000 pg/ml) into wells **A1** and **A2**. Transfer 100 µl from wells **A1** and **A2** in to wells **B1** and **B2**.
   - Mix wells **B1** and **B2** by pipetting.
   - Transfer 100 µl from well **B1** to **C1** and **B2** to **C2**.
   - Continue this serial dilution process to wells **G1** and **G2**. After mixing, discard 100 µl of solution from wells **G1** and **G2**.
   - Do not add standard to wells **H1** and **H2**. These will serve as blanks.

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Table 1. Set up of a 96-well microtiter plate.

	Standard	Standard	Your Samples									
	1	2	3	4	5	6	7	8	9	10	11	12
A	4000 pg/ml	4000 pg/ml*	-	-	-	-	-	-	-	-	-	-
B	2000 pg/ml	2000 pg/ml	-	-	-	-	-	-	-	-	-	-
C	1000 pg/ml	1000 pg/ml	-	-	-	-	-	-	-	-	-	-
D	500 pg/ml	500 pg/ml	-	-	-	-	-	-	-	-	-	-
E	250 pg/ml	250 pg/ml	-	-	-	-	-	-	-	-	-	-
F	125 pg/ml	125 pg/ml	-	-	-	-	-	-	-	-	-	-
G	62.5 pg/ml	62.5 pg/ml	-	-	-	-	-	-	-	-	-	-
H	Blank	Blank	-	-	-	-	-	-	-	-	-	-

**Sensitivity: 4000 pg/ml is a recommended starting dilution. By reducing the starting concentration, researchers can determine the lowest detection limit of IL-33 protein in the test samples.*

4. **Samples:** Pipet 100 µl of positive and negative controls and 100 µl test samples into the appropriate wells*. Seal the plate and incubate for 2 h at RT on a shaker. Samples may be diluted or serially diluted using 1X Blocking Buffer. **Users need to empirically determine the optimal concentrations of their test samples so that the readings fall within the curve of the protein standard.*

5. **Washing:** Remove samples and controls and wash 4X with 300 µl of 1X Wash Buffer. Tap plate several times upside down to remove residual Wash Buffer after final wash.

6. **Detecting Antibody:** Dilute 120 µl of 100X Detecting Antibody (KC-542-2) in 12 ml of 1X Blocking Buffer and add 100 µl 1X Detecting Antibody to each well. Seal the plate and incubate for 1 h at RT on a shaker.

7. **Washing:** Remove antibody solution and wash wells 4x with 300 µl of 1X Wash Buffer. Tap plate upside down to remove residual Wash Buffer after final wash.

8. **Secondary:** Dilute 2.4 µl of HRP-Conjugated Streptavidin (KC-143) in 12 ml of 1X Blocking Buffer (for one plate). Add 100 µl of diluted secondary to each well. Seal the plate and incubate for 30min at RT on a shaker.

9. Remove the secondary antibody and wash thoroughly (5X) with 300 µl of Wash Buffer letting the solution sit in wells briefly between each wash. This ensures a thorough wash and lower background. Tap plate upside down several times to remove any residual Wash Buffer.

10. **ABTS:** Add 100 µl of ABTS Substrate (KC-144) to each well. Incubate the plate at RT for 15 to 30 min. Read the color development at 405 nm.

Note: *Incubation time with ABTS Substrate may be increased or decreased depending on the concentration of samples. Most plate readers have a maximum reading of 2.0-3.0.*

VII. HUMAN IL-33 ACTIVELISA™ TEST DATA

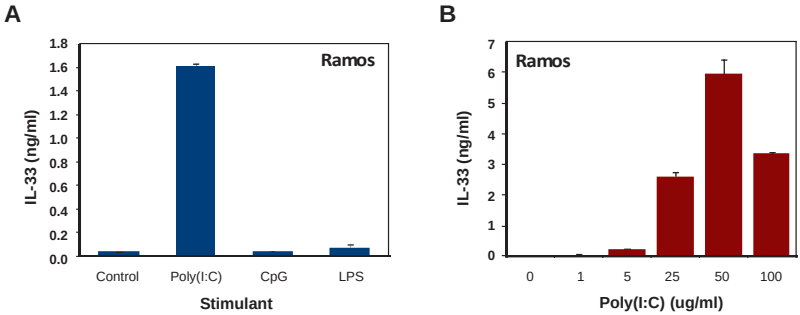

Figure 1. Induction of human IL-33 in Ramos cells. Ramos cells were treated with Poly(I:C) (IMGENEX, IMG-2203; 80 ug/ml), CpG (IMGENEX, IMG-2209Hpt; 20 ug/ml) or LPS (IMGENEX, IMG-2204; 2 ug/ml) [A] as well as were treated with increasing amount of Poly(I:C) (0, 1, 5, 25, 50 and 100 ug/ml) [B]. Sixteen hours later, cell culture media were harvested and secreted IL-33 were assessed by hIL-33 ELISA.

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VIII. TROUBLESHOOTING IL-33 ACTIVELISA™

Problem	Probable Cause	Suggestion
No signal	Failure to add all components.	Prepare a check-list and add the components in the correct order.
Low signal	Not enough supernatant per well.	Check the protein concentration. Add more Sup.
High background	Improper blocking.	Incubate with blocking buffer as recommended in the manual.
	Wells are not washed enough.	Wash plates thoroughly after each incubation.

NOTES

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