Product Manual

IL-33 soluble (human), detection set

[Interleukin-33, Soluble (human) Detection Set]

For Research Use Only

APO-54N-025

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INTENDED USE

The Interleukin-33 (IL-33) soluble (human) detection set is to be used for the *in vitro* quantitative determination of soluble human IL-33 in biological fluids. This Detection Set is for research use only.

INTRODUCTION

Interleukin-1 (IL-1) family members, such as IL-1α/β and IL-18 play important roles in host defense, immune regulation, neuronal injury and inflammation (1, 2). Recently, a new member of this family, IL-33, also named Nuclear Factor from High Endothelial Venules (NF-HEV) (3), has been reported to be the specific ligand for ST2 (4), an orphan receptor of the IL-1 receptor family (5). Structurally, IL-33 is closest to IL-18, rather than IL-1β. The human IL-33 is expressed with a prodomain (aa 1-111) that is cleaved by caspase-1 to produce a 18kDa mature protein (112-270) (6). The stimulation of ST2 by IL-33 induces the NF-κB and MAPK pathways. IL-33 is synthesized in response to cytokines such as IL-1β (but also TNFα) and plays an important role in T_H2-associated immunology, by triggering an increase secretion of IL-5 and IL-13 from polarized T_H2 Th1 cells. Overexpression of IL-33 in mice leads to severe pathological changes in mucosal organs.

1. Lee, J. K. et al. Differences in signaling pathways by IL-1beta and IL-18. Proc Natl Acad Sci U S A 101, 8815-20 (2004).
2. Allan, S. M., Tyrrell, P. J. & Rothwell, N. J. Interleukin-1 and neuronal injury. Nat Rev Immunol 5, 629-40 (2005).
3. Baekkevold, E. S. et al. Molecular characterization of NF-HEV, a nuclear factor preferentially expressed in human high endothelial venules. Am J Pathol 163, 69-79 (2003).
4. Schmitz, J. et al. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. Immunity 23, 479-90 (2005).
5. Brint, E. K. et al. ST2 is an inhibitor of interleukin 1 receptor and Toll-like receptor 4 signaling and maintains endotoxin tolerance. Nat Immunol 5, 373-9 (2004).
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PRINCIPLE OF PROCEDURE

This assay is a sandwich Enzyme-Linked Immuno Sorbent Assay (ELISA) developed for the direct measurement of human IL-33 [IL-33 (human)] in biological fluids. A polyclonal antibody specific for IL-33 (human) is coated onto the wells of a microtiter plate. Samples and standards of IL-33 are pipetted into the wells for binding to the coated antibody. After extensive washing to remove unbound compounds, IL-33 is recognized by the addition of a biotinylated monoclonal antibody specific for IL-33 (human). After removal of excess biotinylated antibody, streptavidin-peroxidase is added. Following a final washing, peroxidase activity is quantified using the substrate 3,3',5,5'-tetramethylbenzidine (TMB). The intensity of the color reaction is measured at 450 nm after acidification and is directly proportional to the concentration of IL-33 in the samples.

MATERIALS PROVIDED

- 3 vials Standard (*lyophilized*) (625pg each) Product No. 80-2353
- 1 vial Coating Antibody (260µL) Product No. 80-2106
- 1 vial Detection Antibody (60µL) Product No. 80-2107

MATERIALS REQUIRED

- PBS: 137mM NaCl, 2.7mM KCl, 8.1mM Na_2HPO_4, 1.5mM KH_2PO_4, pH 7.2, 0.2µM filtered
- Wash Buffer: 0.1%Tween® 20 in PBS
- Blocking Buffer: 10mM Na_2HPO_4, 15mM NaCl, 1% Sucrose, 0.1% Tween 20, pH 8
- Diluent Buffer: 2% BSA in PBS, 0.2µM filtered.
- 1N Hydrochloric Acid (HCl)
- Tetramethylbenzidine substrate kit (NEOGEN, code 308177)
- Streptavidin-peroxidase (Jackson Immunoresearch, N° 016-030-084)
- HPLC grade water
- Costar flat-bottom, high binding 96 well plate, code 92592

PRODUCT SPECIFICATION

Number of Assays: This Detection Set contains sufficient materials to run 5 x ELISAs on 96-well plates.

Specificity: The antibodies used in this detection Set are specific for the measurement of natural and recombinant human IL-33. They do not cross-react with mouse IL-33.

Sensitivity: 5 pg/mL (range 0 to 500pg/ml)

Stability: Coating and Detection Antibodies are stable for at least 6 months when stored at +4°C. **The standard must be stored at -20°C.**

GENERAL ELISA PROTOCOL

Preparation of Reagents

1. Dilute the desired amount of Coating Antibody 1:200 in PBS without carrier protein and use it fresh.
2. Dilute the desired amount of Detection Antibody 1:1000 in Diluent Buffer and use it fresh.
3. Reconstitute the Standard Protein with 1250µL Wash Buffer to obtain a concentration 50pg/ml. **After reconstitution, prepare aliquots and store the reconstituted standard at -20°C! Avoid freeze/thaw cycles!** A seven point standard curve using 2-fold serial dilutions in Wash Buffer is recommended. Suggested standard points are 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, 31.25pg/ml, 15.6pg/mL and 7.8pg/ml.

   The diluted standards should be used within 1 hour of preparation.
4. If measuring serum or cell culture supernatant, dilute samples in Wash Buffer.
5. To reduce interference from rheumatoid factor (RF) in the serum, Ig can be cleared by treating serum dilutions with 0.05 volume of protein G-Sepharose (Amersham Biosciences) twice for one hour at 4°C before pelleting the beads and collecting supernatants (precleared sera).

Plate Preparation

1. Coat the wells by adding 100µL/well of 1:200 Coating Antibody diluted in PBS to a 96-well ELISA microplate (Costar flat-bottom, high binding 96 well plate is suggested). Cover the plate with plastic film and leave overnight at 4°C.
2. Aspirate the coated wells and add 300µL of Wash Buffer using a multichannel pipette or autowasher. Repeat the process for a total of five washes. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.
3. Block plates by adding 300µL of Blocking Buffer at RT°C for 2 hr.
4. Repeat the aspiration/wash as in step 2 for a total of five washes.

Assay Procedure

1. Add a total of 100µL/well diluted serum, diluted culture supernatant or Standard Protein serial dilutions in Wash Buffer to the plate.
2. Cover the plate with plastic film and incubate for one hour at RT.
3. Repeat the aspiration/wash as in step 2 of “Plate Preparation” for a total of five washes.
4. Add 100µL/well of the diluted (1:1000) Detection Antibody.
5. Cover the plate with plastic film and incubate for one hour at RT.
6. Repeat the aspiration/wash as in step 2 of “Plate Preparation” for a total of five washes.
7. Add 100 µL to each well of the diluted HRP Labeled Streptavidin (from Jackson Immunoresearch, diluted 1/10’000).
8. Cover the plate with plastic film and incubate for 30 min at RT.
9. Aspirate the coated wells and add 300 µL of Wash Buffer (Wash Buffer 1X) using a multichannel pipette or autowasher. Repeat the process for a total of five washes. After the last wash, complete removal of liquid is essential for good performance.
10. Substrate development is conducted by addition of 100µL to each well of ready-to-use tetramethylbenzidine (TMB) (NEOGEN).
11. Stop the reaction by adding 50µL of 1N HCl. Tap the plate gently to ensure thorough mixing.
12. Measure the OD at 450nm in an ELISA reader.
13. Measure absorbance at 550nm and subtract these values from those obtained at 450nm to correct for optical imperfections in the microplate. If absorbance at 550nm is not possible, measure the absorbance at 450nm only.

    Note: When the 550 nm measurement is omitted, absorbance values will be higher.

TECHNICAL HINTS AND LIMITATIONS

- Once reagents have been added to the plate, DO NOT let the plate dry at any time during the assay.
- Be sure no sodium azide is present in this assay, as this inhibits HRP enzyme activity.
- It is recommended that all standards and samples be assayed in duplicate.
- Avoid microbial contamination of reagents and equipment. Automated plate washers can become contaminated thereby causing assay variability. Buffers containing a large quantity of protein should be made under sterile conditions and stored at 2-8°C or be prepared fresh daily.
- Vigorous plate washing is essential.
- Avoid exposing reagents to excessive heat or light during storage and incubation.
- Wear gloves while performing the assay to avoid contact with samples and reagents. Please follow proper disposal procedure.

CALCULATION OF RESULTS

- Average the duplicate readings for each standard, control and sample and subtract the average blank value.
- Generate the standard curve by plotting the average absorbance obtained for each standard concentration on the vertical (Y) axis vs. the corresponding IL-33 concentration (pg/ml) on the horizontal axis (**TYPICAL DATA**).
- Calculate results using graph paper or curve-fitting statistical software. The amount of IL-33 in each sample is determined by interpolating for the absorbance value (Y axis) using the standard curve.
- If the test sample was diluted, multiply the interpolated value by the dilution factor to calculate pg/mL of IL-33 in the sample.

TYPICAL DATA

The following data are obtained using the different concentrations of standard as described in this protocol:

Standard IL-33 (pg/ml)	Optical Density (mean)
0	0.086
7.8	0.114
15.6	0.150
31.25	0.211
62.5	0.330
125	0.576
250	0.986
500	1.808

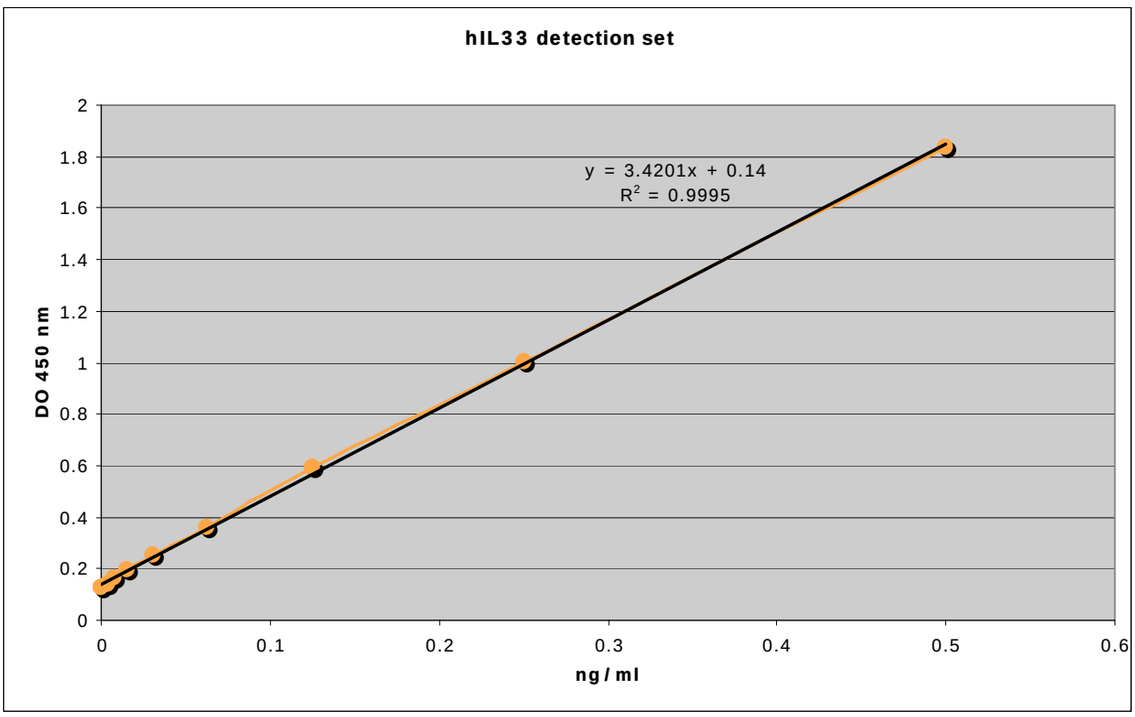

Figure: Standard curve

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GLOBAL HEADQUARTERS
Enzo Life Sciences Inc.
10 Executive Boulevard
Farmingdale, NY 11735
Toll-Free:1.800.942.0430
Phone:631.694.7070
Fax:631.694.7501
info-usa@enzolifesciences.com

EUROPE/ASIA
Enzo Life Sciences (ELS) AG
Industriestrasse 17
CH-4415 Lausen
Switzerland
Phone:+41/0 61 926 89 89
Fax:+41/0 61 926 89 79
info-ch@enzolifesciences.com

For local distributors and detailed product information visit us online:
www.enzolifesciences.com