

Human IL1RL1 ELISA Kit

Sandwich ELISA

Catalog EL-213-91201

INTRODUCTION

IL1RL1, also known as ST2, is a receptor for the cytokine interleukin-33 and, together with the shared coreceptor IL1RAP, triggers an intracellular cascade involving MyD88, IRAK1/4, and TRAF6 that culminates in NF- κ B and MAPK activation and a downstream Th2-skewed inflammatory response. The gene produces two principal protein forms from alternative promoters: a membrane-anchored receptor that transmits IL-33 signaling on immune and stromal cells, and a truncated, secreted form, soluble ST2, that circulates in blood and acts as a decoy, mopping up free IL-33 before it can engage the membrane receptor. Because cardiac and vascular stress raise circulating soluble ST2, it has become an established biomarker used alongside natriuretic peptides for risk stratification in heart failure and other cardiovascular conditions. Genetic variation in IL1RL1 has also been associated with blood eosinophil counts and asthma susceptibility, reflecting its broader role in type 2 immune responses.

PRINCIPLE OF THE ASSAY

This kit utilizes a sandwich enzyme immunoassay format to detect Human IL1RL1. The microtiter plate wells are pre-coated with a capture antibody specific to Human IL1RL1, which binds the target protein present in standards or samples. A biotin-conjugated detection antibody directed against a second epitope of Human IL1RL1 is then added, followed by incubation with Avidin conjugated to Horseradish Peroxidase (HRP). Upon addition of TMB substrate, wells containing Human IL1RL1, the biotin-conjugated detection antibody, and HRP-conjugated Avidin undergo a colorimetric change proportional to the amount of analyte present. The enzymatic reaction is stopped by the addition of a sulphuric acid stop solution, and absorbance is measured at 450 nm ($\pm$ 10 nm). IL1RL1 concentrations in unknown samples are then calculated by comparing their optical density values against a standard curve.

MATERIALS AND STORAGE

Store kit components at -20°C unless specified otherwise. DO NOT USE past kit expiration date. Some vials contain a small amount of reagents. Spin tubes on pulse setting prior to opening.

Each kit includes:	Units
Pre-Coated Microplate	12 strips x 8 wells
Standard (Lyophilized)	2 vials
Biotinylated Antibody (100 $\times$)	120 μ L
Streptavidin-HRP (100 $\times$)	120 μ L
Standard/Sample Diluent Buffer	20 mL
Biotinylated Antibody Diluent	12 mL
HRP Diluent	12 mL
Wash Buffer (25 $\times$)	20 mL
TMB Substrate Solution	10 mL
Stop Reagent	6 mL
Plate Covers	2 pieces
<i>Do not mix or substitute reagents with those from other lots.</i>	

MATERIALS AND INSTRUMENTS REQUIRED BUT NOT SUPPLIED

- Precision pipettes calibrated to deliver 5-1000 μ L
- Multi-channel pipette calibrated to deliver 50-200 μ L
- Plate shaker
- Disposable tips
- Vortex-Mixer
- Distilled or de-ionized water
- Microplate reader capable of reading 450nm with background subtraction at 620nm

SAFETY PRECAUTIONS

- The test protocol must be followed strictly.
- All reagents containing human material should be handled as if potentially infectious. Operators should wear gloves and protective

clothing when handling any patient sera or serum based products.

- The kit reagents contain antimicrobial agents, acid and 3,3',5,5'-tetramethylbenzidine. Avoid contact with the skin and eyes. Rinse immediately with plenty of water if any contact occurs.
- Any liquid that has been brought into contact with potentially infectious material has to be discarded in a container with a disinfectant. Disposal must be performed in accordance with local regulations.
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- Only trained laboratory personnel should execute this test.

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PREPARATION OF REAGENTS

Prepare only the appropriate amount of required reagent on the day of use. Store all reagents as per instructions stated on the label.

- 1. Wash Buffer (1X) Preparation: Dilute the 25X wash buffer concentrate 1:25 with double-distilled Water.**
- 2. Detection Reagent (1X) Preparation: Dilute the biotinylated antibody and streptavidin-HRP concentrates 100-fold with Biotinylated Antibody Diluent and HRP Diluent, respectively, immediately before use.**
- 3. Preparation of Calibrators: Prepare calibrators by serial dilution from a 4000 down to 62.5 (plus a 0 blank), per the source manual.**

Sol'n ID	Source	Source Vol (μL)	Cal. Diluent (μL)	Final Vol (μL)	Final Conc. (ng/mL)
Std 1	Stock Standard	500	500	1000	4000
Std 2	Std 1	500	500	1000	2000
Std 3	Std 2	500	500	1000	1000
Std 4	Std 3	500	500	1000	500
Std 5	Std 4	500	500	1000	250
Std 6	Std 5	500	500	1000	125
Std 7	Std 6	500	500	1000	62.5
Blank	Std 7	500	500	1000	0

SPECIMEN STORAGE

This kit is compatible with EDTA-plasma, heparin-plasma and serum samples. Samples can be stored at or below -20°C for up to 1 year.

ASSAY PROCEDURE

1. After the kit is equilibrated at room temperature, add 100 μL of Standard Working Buffer (gradually diluted according to the instructions) or 100 μL of sample to each well, and incubate at 37°C for 80 minutes.

2. Discard the liquid in the plate, add 200 μL 1× Wash Buffer to each well, and wash the plate 3 times. After pat it dry against clean absorbent paper, add 100 μL Biotinylated Antibody Working Solution (1×) to each well, incubate at 37°C for 50 minutes.

3. Discard the liquid in the plate, add 200 μL 1× Wash Buffer to each well, and wash the plate 3 times. After pat it dry against clean absorbent paper, add 100 μL 1× Streptavidin-HRP Working Solution to each well, incubate at 37°C for 50 minutes.

4. Discard the liquid in the plate, add 200 μL 1× Wash Buffer to each well, and wash the plate 5 times. After pat it dry against clean absorbent paper, add 90 μL TMB Substrate Solution to each well, incubate at 37°C for 20 minutes in the dark.

5. Add 50 μL Stop Solution to each well, shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm immediately, calculation of the results.

CALCULATIONS AND RESULTS

- Construct a standard curve by plotting the absorbance obtained from each standard against concentration. Use a 4 or 5 parameter curve fit. Alternatively a log-log curve fit may be used.
- The concentration of the unknowns can be read directly from this standard curve using the absorbance value for each sample.

3. Any sample undiluted or diluted still reading greater than the highest standard should be diluted appropriately and retested. If the samples have been diluted, the concentration determined from the standard curve must be multiplied by the dilution factor.

PERFORMANCE CHARACTERISTICS

Precision: Intra-assay CV < 8%. Inter-assay CV < 10%.

Detection Limit: 21.5 pg/mL

Recovery: Serum (n = 5): 82-94% (avg 88%); EDTA plasma (n = 5): 85-97% (avg 91%); Heparin plasma (n = 5): 81-93% (avg 87%)

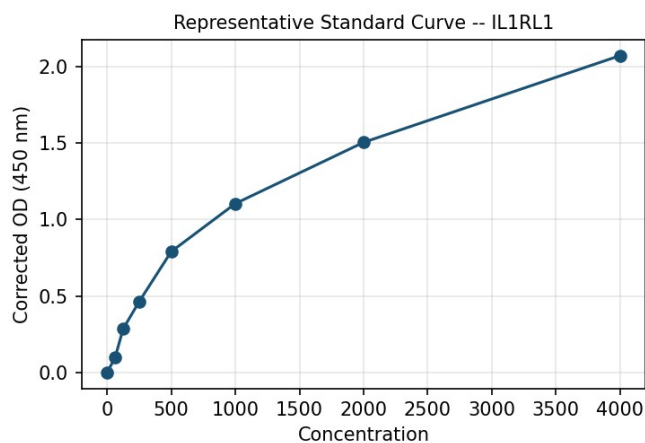
Specificity: This assay has high sensitivity and excellent specificity for detection of Human IL1RL1. No significant cross-reactivity or interference between Human IL1RL1 and analogues was observed.



This kit employs a sandwich enzyme immunoassay technique to quantify Human IL1RL1 in biological samples.

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SAMPLE STANDARD CURVE



Representative standard curve shown for illustration only. This curve should not be used to calculate unknown sample concentrations -- run a new standard curve with each assay.

ORDERING INFORMATION

Please visit www.affinityimmuno.com to order this product. Visa, Mastercard, AMEX and PayPal are accepted in our online store.

Your order will be processed immediately and you will be notified with a delivery timeframe.