This kit employs a sandwich enzyme immunoassay technique to quantify IL-36beta in biological samples.

Human IL-36beta ELISA Kit
Sandwich ELISA
Catalog EL-213-912363

INTRODUCTION

IL-36 beta belongs to the interleukin-1 superfamily and, like its paralogs, is predicted to fold into the family's characteristic 12-stranded beta-trefoil structure; its gene sits within a cluster of nine IL-1-related genes on chromosome 2. Acting through the IL-36 receptor, IL1RL2, paired with the shared accessory chain IL1RAP, IL-36 beta activates NF-kB and MAPK signaling to stimulate production of interleukin-6 and interleukin-8 in synovial fibroblasts, cartilage cells, and adipocytes, and it induces antimicrobial peptides and matrix-degrading enzymes in skin. Through effects on keratinocytes, dendritic cells, and T cells it contributes to epithelial barrier inflammation, and dysregulated IL-36 beta activity has been tied to psoriatic disease, including generalized pustular psoriasis, as well as inflammatory bowel disease and rheumatoid arthritis. Because it is highly related in sequence and function to IL-36 alpha and gamma, antibody pairs with minimal cross-reactivity to those paralogs are needed to measure it specifically.

PRINCIPLE OF THE ASSAY

This kit uses a sandwich enzyme immunoassay format. Microtiter plate wells are pre-coated with a capture antibody specific to IL-36beta (Human IL1h), which binds the target analyte present in standards or samples added to each well. A biotin-conjugated detection antibody, also specific to IL-36beta, is then introduced to form a sandwich complex on any well containing the analyte. Avidin conjugated to horseradish peroxidase (HRP) is subsequently added and binds the biotin, completing the detection assembly. Upon addition of TMB substrate solution, only wells containing IL-36beta, the biotin-conjugated detection antibody, and avidin-HRP produce a colorimetric signal. The enzymatic reaction is stopped by the addition of sulphuric acid solution, and absorbance is measured spectrophotometrically at 450 nm (± 10 nm). IL-36beta concentrations in the samples are determined by comparing sample optical densities 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×)	120 µL
Streptavidin-HRP (100×)	120 µL
Standard/Sample Diluent Buffer	20 mL
Biotinylated Antibody Diluent	12 mL
HRP Diluent	12 mL
Wash Buffer (25×)	20 mL
TMB Substrate Solution	10 mL
Stop Reagent	6 mL
Plate Covers	2 pieces
Do not mix or substitute reagents with those from other lots.	

MATERIALS AND INSTRUMENTS REQUIRED BUT NOT SUPPLIED

- Precision pipettes calibrated to deliver 5-1000µL
- Multi-channel pipette calibrated to deliver 50-200µL
- 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 500 down to 7.82 (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	500
Std 2	Std 1	500	500	1000	250
Std 3	Std 2	500	500	1000	125
Std 4	Std 3	500	500	1000	62.5
Std 5	Std 4	500	500	1000	31.25
Std 6	Std 5	500	500	1000	15.63
Std 7	Std 6	500	500	1000	7.82
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

1. 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.
2. 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: 2.6 pg/mL

Recovery: Serum (n = 5): 77-95% (avg 86%); EDTA plasma (n = 5): 94-106% (avg 100%); Heparin plasma (n = 5): 80-97% (avg 88%)

Specificity: This assay has high sensitivity and excellent specificity for detection of Human IL1h. No significant cross-reactivity or interference between Human IL1h and analogues was observed.

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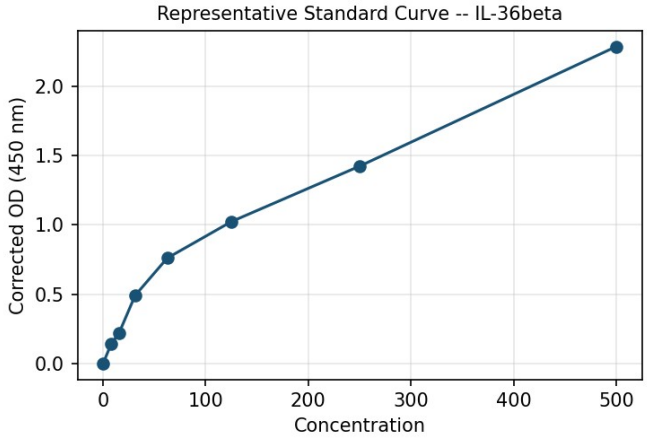
SAMPLE STANDARD CURVE



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.