This kit employs a sandwich enzyme immunoassay technique to quantify IL-18BP in biological samples.

Human IL-18BP ELISA Kit
Sandwich ELISA
Catalog EL-213-91218

INTRODUCTION

IL-18BP is a small, constitutively secreted protein built around a single immunoglobulin-like domain that circulates freely in blood and acts as a natural brake on interleukin-18 signaling. By binding IL-18 before it can engage its cell-surface receptor, it blunts the downstream burst of interferon-gamma and dampens the type of T-helper-1-skewed immune response that IL-18 would otherwise drive, and it may also shape type 2 (Th2) cytokine output. The protein is produced constitutively by mononuclear immune cells and is also strongly expressed in tissues such as heart, lung, spleen, and placenta, and several splice-derived isoforms of differing size exist, though only one has been shown to bind IL-18 effectively. Altered levels of IL-18BP have been reported in inflammatory bowel conditions such as Crohn's disease, and rare inherited defects in the gene have been linked to a severe, recessive form of fulminant viral hepatitis.

PRINCIPLE OF THE ASSAY

This kit uses a sandwich enzyme immunoassay format to measure Human IL-18BP. The microtiter plate wells are pre-coated with a capture antibody specific to Human IL-18BP, which binds the target analyte present in standards or samples upon addition to the wells. A biotin-conjugated detection antibody, also specific to Human IL-18BP, is then introduced and recognizes a second epitope on the captured analyte. Avidin conjugated to horseradish peroxidase (HRP) is subsequently added to each well, where it binds the biotin moiety of the detection antibody. Following addition of TMB substrate solution, only wells containing Human IL-18BP, the biotin-conjugated detection antibody, and avidin-HRP undergo a colorimetric change. The enzymatic reaction is halted by the addition of a sulphuric acid stop solution, and absorbance is measured spectrophotometrically at 450 nm (± 10 nm). IL-18BP concentrations in unknown samples are determined by interpolating 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×)	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.
- Disposal must be performed in accordance with local regulations.
- Only trained laboratory personnel should execute this test.

This kit employs a sandwich enzyme immunoassay technique to quantify IL-18BP in biological samples.

Human IL-18BP ELISA Kit
Sandwich ELISA
Catalog EL-213-91218

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 100 down to 1.57 (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	100
Std 2	Std 1	500	500	1000	50
Std 3	Std 2	500	500	1000	25
Std 4	Std 3	500	500	1000	12.5
Std 5	Std 4	500	500	1000	6.25
Std 6	Std 5	500	500	1000	3.13
Std 7	Std 6	500	500	1000	1.57
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: 0.52 ng/mL

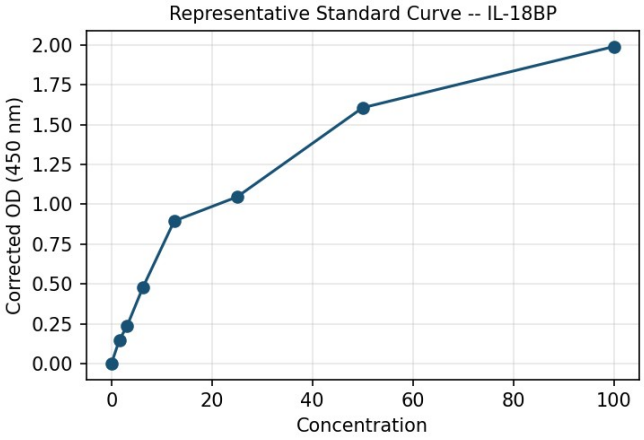
Recovery: Serum (n = 5): 83-99% (avg 95%); EDTA plasma (n = 5): 80-96% (avg 88%); Heparin plasma (n = 5): 87-99% (avg 93%)

Specificity: This assay has high sensitivity and excellent specificity for detection of Human IL18BP. No significant cross-reactivity or interference between Human IL18BP and analogues was observed.

This kit employs a sandwich enzyme immunoassay technique to quantify IL-18BP in biological samples.

Human IL-18BP ELISA Kit
Sandwich ELISA
Catalog EL-213-91218

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.