LTB4 ELISA Kit

Catalog Number: EHLTB4

Product description

A competitive immunoassay for the quantitative determination of Leukotriene B_4 (LTB_4) in biological fluids. The assay is based on the competition between LTB_4 in the standard or sample and Alkaline Phosphatase conjugated LTB_4 (LTB_4-AP) for a limited amount of LTB_4 monoclonal antibody bound to an Anti-Rabbit IgG precoated 96-well plate. As the concentration of LTB_4 in the sample increases, the amount of LTB_4-AP captured by the coating antibody decreases. Thus, there is an inverse relationship between optical density (OD) and the amount of analyte in the sample.

Contents and storage

All components of this kit, except the conjugate, are stable at 4°C until the kit's expiration date. The conjugate must be stored at -20°C.

Description	Size
Antibody Coated Plate, 96-well plate	1 plate
Reagent Diluent	27 mL
LTB_4 Antibody	5 mL
LTB_4-AP Conjugate	5 mL
LTB_4 Standard (120,000 pg/mL LTB_4)	0.5 mL
20X Wash Buffer	27 mL
Substrate Solution	20 mL
Stop Solution	5 mL
Plate Sealer	1 each

Additional required materials

- Deionized or distilled water
- Precision pipettes (for volumes between 5 µL and 1,000 µL)
- Repeater pipettes (for dispensing 50 µL and 200 µL)
- Disposable beakers for diluting buffer concentrates
- Graduated cylinders
- 37°C incubator
- Microplate shaker
- Absorbent lint free paper for blotting
- Microplate reader capable of reading at 405 nm, preferable with correction between 570 and 590 nm.
- 2M hydrochloric acid, deionized water, ethanol, hexane and ethyl acetate
- 200 mg C18 reverse phase extraction columns

General guidelines

- Do not mix components from different kit lots or use reagents beyond the kit expiration date.
- Pre-rinse the pipette tip with the reagent, use fresh pipette tips for each sample, standard or reagent.
- Care must be taken to minimize contamination by endogenous alkaline phosphatase (AP). Contaminating AP activity, especially in the substrate solution, may lead to high blanks.
- Care should be taken not to touch pipette tips and other items that are used in the assay with bare hands.
- Allow kit components to come to room temperature for at least 30 minutes before use.
- Prior to addition of substrate, ensure that there is no residual wash buffer in the wells. Any remaining wash buffer may cause variation in assay results.

Assay compatibility

- The LTB_4 ELISA is compatible with LTB_4 samples in a wide range of matrices after dilution in Reagent Diluent.
- The assay is also compatible with samples in the majority of tissue culture media, including those containing fetal bovine serum.
- Samples containing rabbit IgG may interfere with the assay.

Prepare 1X Wash Buffer

1. Allow the 20X Wash Buffer to reach room temperature and mix to redissolve any precipitated salts.
2. Dilute 5 mL of 20X Wash Buffer with 95 mL of deionized or distilled water. Label as 1X Wash Buffer.

   The diluted buffer is stable for up to 3 months at room temperature.

Sample preparation guidelines

- Add 10 µg/mL prostaglandin synthetase inhibitors (e.g., indomethacin or meclofenamic acid) to tissue homogenate, urine and plasma samples.
- Perform extraction procedure on samples with very low levels of LTB_4 (see "Extraction procedure example").
- Because conditions may vary, it is recommended that each investigator determine the optimal dilution to be used for each application.

Sample type	Dilution
Tissue culture media	Neat
Human urine	Neat
Human saliva	>1:4
Porcine EDTA plasma	1:2 to 1:4

Extraction procedure example

1. Add 2M HCl to acidify plasma, urine, or tissue homogenate to pH 3.5. Use approximately 50 µL of HCl per 1 mL of plasma. Incubate at 2°C to 8°C for 15 minutes. Centrifuge samples for 2 minutes to remove any precipitate.
2. Wash the C18 reverse phase column (200 mg) with 10 mL of ethanol followed by 10 mL of deionized water.
3. Apply the sample under a slight positive pressure to obtain a flow rate of about 0.5 mL/minute. Wash the column with 10 mL of water, followed by 10 mL of 15% ethanol, and finally 10 mL hexane. Elute the sample from the column by addition of 10 mL ethyl acetate.
4. If proceeding immediately to analysis, evaporate solvent in samples under a nitrogen stream. Add at least 250 µL of Reagent Diluent to the dried samples. Vortex well, then allow to sit for five minutes at room temperature. Repeat twice more.

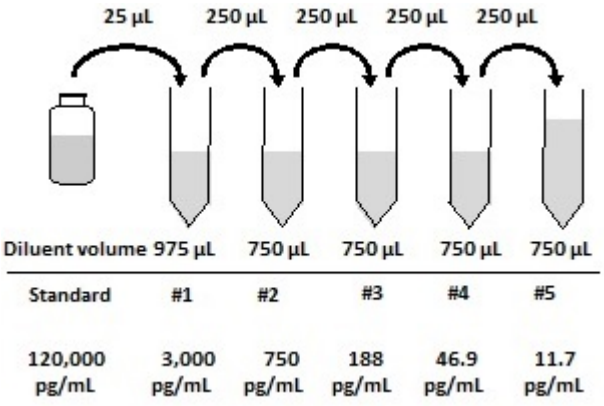
   If performing analysis later, store samples in ethyl acetate at –80°C until the immunoassay is to be run. Evaporate the organic solvent under a stream of nitrogen prior to running the assay and reconstitute as above.

Standard preparation guidelines

- Run standard curves in the appropriate matrix.
- For samples in tissue culture media, dilute standards in the same type of media instead of Reagent Diluent. A small change in binding is associated with running standards and samples in media.
- Use the diluted standards within 60 minutes.

Dilute standards

1. Allow the LTB_4 Standard to warm to room temperature.
2. Label five 12 × 75mm tubes #1 through #5.
3. Add 975 µL of standard diluent (Reagent Diluent or tissue culture media) to Tube #1.
4. Add 750 µL Reagent Diluent to Tubes #2 to #5.
5. Add 25 µL LTB_4 standard to Tube #1 and vortex thoroughly.
6. Add 250 µL of Tube #1 to Tube #2 and vortex thoroughly.
7. Continue to make serial dilutions of the standard as shown in the diagram below.



ELISA procedure

Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store at 2 to 8°C for future use.

Run all standards and samples in duplicate.

1. Add 100 µL of standard diluent (Reagent Diluent or tissue culture media) into the NSB and the B_0 (0 pg/mL Standard) wells.
2. Add 100 µL of Standards #1 through #5 into the appropriate wells.
3. Add 100 µL of the Samples into the appropriate wells.
4. Add 50 µL of Reagent Diluent into the NSB wells.
5. Add 50 µL of the blue LTB_4-AP conjugate into each well, except the Total Activity (TA) wells and blank wells.
6. Add 50 µL of the yellow LTB_4 antibody into each well, except the Blank, TA and NSB wells.

   NOTE: Every well used should be GREEN in color except the NSB wells which should be BLUE. The Blank and TA wells are empty at this point and have no color.
7. Incubate the plate at room temperature on a plate shaker for 2 hours at ~500 rpm.
8. Empty the contents of the wells and wash by adding 400 µL of the 1X Wash Buffer to every well. Repeat the wash 2 more times for a total of 3 washes.
9. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
10. Add 5 µL of the blue LTB_4-AP conjugate to the TA wells.
11. Add 200 µL of the Substrate Solution to every well. Seal the plate and incubate at 37°C for 2 hours.
12. Add 50 µL of Stop Solution to every well and read the plate immediately.
13. Blank the plate reader against the Blank wells, read the optical density at 405 nm preferably with correction between 570 and 590 nm. If the plate reader is not able to be blanked against the Blank wells, manually subtract the mean optical density of the Blank wells from all readings.

Calculations

Several options are available for the calculation of the concentration of LTB_4 in the samples. It is recommended that the data be analyzed by a 4 parameter logistic curve fitting program. If data reduction software is not available, the concentration of the LTB_4 can be calculated:

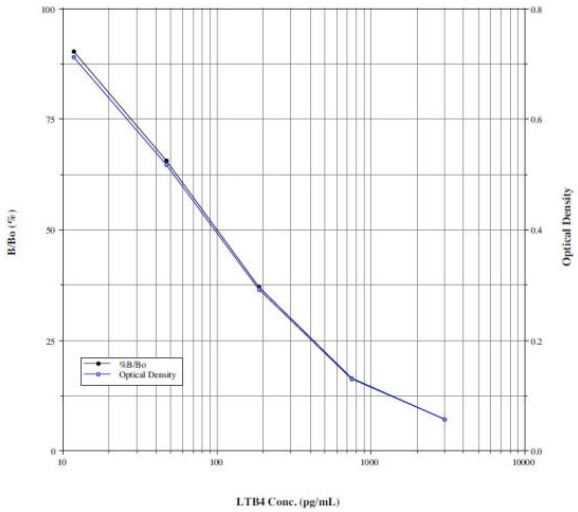
1. Calculate the average net Optical Density (OD) bound for each standard and sample by subtracting the average Blank OD from the average OD for each standard and sample.

   Average Net OD = Average OD - Average NSB OD
2. Calculate the binding of each pair of standard wells as a percentage of the maximum binding wells (Bo), using the following formula:

   Percent Bound = (Net OD/Net Bo OD) × 100
3. Plot Percent Bound versus LTB_4 concentration for each standard. Approximate a straight line through the points. The concentration of LTB_4 in the unknowns can be determined by interpolation.

Typical standard curve

A typical standard curve is shown below. This curve must not be used to calculate LTB_4 concentrations; a standard curve must be run with every assay.



Performance characteristics

The following parameters for this kit were determined using the guidelines listed in the National Committee for Clinical Laboratory Standards (NCCLS) Evaluation Protocols.

Sensitivity

The minimum detectable dose of LTB_4 is <5.63 pg/mL. This was determined by adding two standard deviations to the mean O.D. obtained from the average OD bound for 20 wells run as B_0, compared to the average OD for 20 wells run with Standard #5.

Linearity

A sample containing 1,000 pg/mL LTB_4 was serially diluted 1:2 in Reagent Buffer and measured in the assay. Linear regression analysis of samples versus the expected concentration yielded a line with a slope of 0.961 and correlation coefficient of 0.999.

Precision

Intra-assay precision was determined by taking samples containing low, medium and high concentrations of LTB_4 and running these samples multiple times (n=16) in the same assay. Inter-assay precision was determined by measuring them in multiple assays (n=8).

The precision numbers listed below represent the percent coefficient of variation for the concentrations of LTB_4 determined in these assays as calculated by a 4 parameter logistic curve fitting program.

Intra-assay	LTB_4 (pg/mL)	%CV
Low	305	6.0
Medium	607	6.8
High	1,078	5.9

Inter-assay	LTB_4 (pg/mL)	%CV
Low	99	15.7
Medium	308	16.5
High	507	5.0

Cross-reactivity

The cross-reactivities for a number of related compounds were determined by dissolving the cross reactant in Reagent Diluent at concentrations from 40,000 to 0.4 pg/mL. These samples were then measured in the LTB_4 assay and the measured LTB_4 concentration at 50% B/B_0 calculated. The % cross reactivity was calculated by comparison with the actual concentration of cross reactant in the sample and expressed as a percentage.

Compound	Cross Reactivity
LTB_4	100%
6-trans-12-epi-LTB_4	5.50%
6-trans-LTB_4	4.90%
12-epi-LTB_4	0.94%
PGE_2, $PGF_{2\alpha}$, 20-OH-LTB_4, 20-COOH-LTB_4, LTC_4, LTD_4, LTE_4, 5(S)-HETE, 12(S)-HETE, 15(S)-HETE	<0.2%

Sample recovery

LTB_4 concentrations were measured in a variety of different samples including tissue culture media, human saliva and urine, and porcine plasma. For samples in tissue culture media, ensure that the standards have been diluted into the same media. LTB_4 was spiked into the undiluted samples of these media, which were then diluted with the appropriate kit Reagent Diluent and then assayed in the kit. The following results were obtained:

Sample	% Recovery	Recommended Dilution
Tissue Culture Media	97.3	None
Human Saliva	114.1	≥1:4
Human Urine	96.9	None
Porcine EDTA Plasma	109.6	1:2 - 1:4

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Product label explanation of symbols and warnings

REF	Catalog Number	LOT	Batch code		Temperature limitation		Use by		Manufacturer		Consult instructions for use		Caution, consult accompanying documents

Manufacturer's address: Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria

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