ELISA Kit
Catalog # KHL1761

Human
Leukotriene E_4

www.invitrogen.com
Invitrogen Corporation
542 Flynn Road, Camarillo, CA 93012
Tel: 800-955-6288
E-mail: techsupport@invitrogen.com

TABLE OF CONTENTS

INTRODUCTION

Leukotrienes C_4, D_4, and E_4 (LTC_4, LTD_4, and LTE_4) are collectively referred to as cysteinyl-leukotrienes (CysLTs). LTC_4 is produced in large quantities by activated leukocytes such as mast cells, eosinophils and macrophages. Biosynthesis of LTC_4 is initiated by the conversion of arachidonic acid to LTA_4 by the enzyme 5-lipoxygenase (5-LO) with 5 (S)-HpETE formed as an intermediate. Subsequent conjugation of glutathione to LTA_4 by LTC_4 synthase produces LTC_4.[1-4] Metabolism of LTC_4 to LTD_4 and LTE_4 occurs rapidly in the circulation by stepwise cleavage of glutamate and glycine from the glutathione adduct by α-glutamyltransferase and dipeptidase.[1-4] LTC_4 and LTD_4, are potent mediators of asthma and hypersensitivity acting *via* a pair of G protein-coupled receptors, $CysLT_1$ and $CysLT_2$.[4-8] They induce bronchoconstriction, increase microvascular permeability, and are vasoconstrictors of coronary arteries.[5,9] LTE_4 has weak affinity for the CysLT receptors and therefore low biological activity. However, its presence, typically assessed in urine, indicates the prior existence of LTC_4 and LTD_4. The levels of intact LTE_4 found in urine are approximately 50-80 pg/mL creatinine.[11]

For Research Use Only. CAUTION: Not for human or animal therapeutic or diagnostic use.

READ ENTIRE PROTOCOL BEFORE USE

PRINCIPLE OF THE METHOD

The Invitrogen Human Leukotriene E_4 (LTE_4) ELISA Kit is a competitive immunoassay for the quantitative determination of LTE_4 in biological samples. This assay is based on the competition between LTE_4 and a LTE_4-alkaline phosphatase tracer for a limited amount of LTE_4-specific antiserum. Because of the competition between LTE_4 in the sample and LTE_4 tracer for the LTE_4 antiserum, the signal obtained with the assay will be inversely proportional to the amount of LTE_4 in each sample. This equilibration is performed in the wells of a 96-well plate pre-coated with mouse polyclonal anti-rabbit IgG, which binds all of the LTE_4 antiserum added to the well. After the incubation step, the plate is washed, and a solution of *para*-nitrophenyl phosphate (*p*NPP), a substrate for alkaline phosphatase, is added. The product of this enzymatic reaction has a distinct yellow color which absorbs at 412 nm, allowing quantification of LTE_4 in each sample.

REAGENTS PROVIDED

Note: *This kit should be stored at -80°C and used before the expiration date.*

Reagent	***96 Test Kit***
Leukotriene E_4 Antiserum, lyophilized. Contains 0.05% sodium azide.	1 vial
Leukotriene E_4 AP Tracer, lyophilized. Contains 0.05% sodium azide.	1 vial
Leukotriene E_4 Standard in ethanol. Refer to vial label for quantity and reconstitution volume; 0.5 mL per vial.	1 vial
Mouse Anti-rabbit IgG Coated Plate, 96 wells per plate.	1 plate
Tris Buffer Concentrate. Contains 0.5% sodium azide; 10 mL per vial.	2 vials
Wash Buffer Concentrate; 5 mL per vial.	1 vial
DEA Buffer Concentrate; 2.5 mL per vial.	1 vial
pNPP Tablets.	5 tablets
Plate Cover, adhesive strips.	1

Disposal Note: This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state and local regulations for disposal.

SUPPLIES REQUIRED BUT NOT PROVIDED

1. Ultrapure (18 MΩ) distilled or deionized water.
2. Calibrated adjustable precision pipettes, preferably with disposable plastic tips. (A manifold-channel pipette is desirable for large assays.)
3. An orbital shaker.
4. A plate reader capable of measuring between 405-420 nm.
5. Plate washer: automated or manual (squirt bottle, manifold dispenser, etc.).
6. Glass tubes for diluting and aliquoting standard.
7. Absorbent paper towels.
8. Calibrated beakers and graduated cylinders in various sizes.
9. 1.0 M HCl.
10. Methanol.
11. Hexane.
12. SPE C-18 cartridges.

PROCEDURAL NOTES/LAB QUALITY CONTROL

1. **Microtiter plates should be allowed to come to room temperature before opening the foil bags.** Once the desired number of strips has been removed, immediately reseal the bag and store at 2 to 8°C to maintain plate integrity.
2. Samples should be frozen if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to analysis.
3. If particulate matter is present, centrifuge or filter prior to analysis.
4. All standards, controls and samples should be run in duplicate.

5. Samples that are greater than the highest standard point should be diluted and retested.
6. When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.
7. Cover or cap all reagents when not in use.
8. **Do not mix or interchange different reagent lots from various kit lots**.
9. Do not use reagents after the kit expiration date.
10. Read absorbances within 2 hours of assay completion.
11. In-house controls should be run with every assay. If control values fall outside pre-established ranges, the accuracy of the assay is suspect.
12. All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. ***Never*** insert absorbent paper directly into the wells.

SAFETY

All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.

DIRECTIONS FOR WASHING

Incomplete washing will adversely affect the test outcome. All washing must be performed with *Wash Buffer* provided.

Washing can be performed manually as follows: completely aspirate the liquid from all wells by gently lowering an aspiration tip (aspiration device) into the bottom of each well. Take care not to scratch the inside of the well.

After aspiration, fill the wells with at least 0.4 mL of diluted *Wash Buffer*. Let soak for 15 to 30 seconds, then aspirate the liquid. Repeat as directed under **ASSAY METHOD**. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.

Alternatively, the *Wash Buffer* may be put into a squirt bottle. If a squirt bottle is used, flood the plate with *Wash Buffer*, completely filling all wells. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.

If using an automated washer, the operating instructions for washing equipment should be carefully followed. If your automated washer allows, 30 second soak cycles should be programmed into the wash cycle.

SAMPLE PREPARATION

Purification Procedure:

While the following purification protocol works well for many samples, it is important to be aware that it may not be sufficient for the purification of LTE_4 from all samples.

1. Split samples into two equal parts and place each sample into clean test tubes.
2. Spike one of these sets of samples with LTE_4.
3. Perform the following steps on all samples:
   a. Acidify the sample to pH <4.0 by the addition of 1.0 M HCl.
   b. Prepare a C-18 SPE cartridge by conditioning it first with 5 mL of methanol followed by 5 mL distilled or deionized water.
   c. Apply the sample and allow to flow through the column.
   d. Rinse the column with 5 mL distilled or deionized water, followed by 5 mL hexane. Allow the column to become dry following the hexane wash.
   e. Elute the LTE_4 with 5 mL methanol. If unable to run the assay at this time, store the samples in methanol at -80ºC.
   f. Dry the sample under a stream of nitrogen. Reconstitute the sample in 1x Tris Buffer. Assay both unspiked and spiked samples with the ELISA.

REAGENT PREPARATION AND STORAGE

A. All diluted buffers should be stored at 2 to 8ºC. When stored in this manner, they will be stable for approximately two months.

1. **Tris Buffer.**
   Dilute the contents of one vial of *Tris Buffer Concentrate* with 90 mL of distilled or deionized water. It is common for the concentrated buffer to contain crystalline salts after thawing. It is important to rinse the vial to obtain any salts that may have precipitated.

2. **Wash Buffer.**
   Dilute the 5 mL vial of *Wash Buffer Concentrate* to a final volume of 750 mL with distilled or deionized water.

3. **DEA Buffer.**
   Dilute the 2.5 mL vial of *DEA Buffer Concentrate* to a final volume of 25 mL with distilled or deionized water.

4. **Leukotriene E_4 Standard.**
   *(**Note**: If assaying culture medium samples that have not been diluted with Tris Buffer, the culture medium rather than Tris Buffer should be used for dilution of the standard curve.)*

   Equilibrate a pipette tip in ethanol, by repeatedly filling and expelling. Use the equilibrated pipette tip to transfer 100 µL of the *LTE_4 Standard* into a clean test tube. Dilute with 900 µL distilled or deionized water. The concentration of this stock standard is 100 ng/mL. Label eight glass test tubes #1 through #8. Pipette 900 µL of 1x Tris Buffer into tube #1, and 500 µL into tubes #2 through #8. Transfer 100 µL of the 100 ng/mL standard into tube #1 and vortex to mix. Transfer 250 µL from tube #1 to tube #2. Vortex to mix. Transfer 250 µL from tube #2 to tube #3. Vortex to mix. Continue this process for standard tubes #4 through #8. These diluted standards should be used within twenty-four hours.

B. Dilution of Leukotriene E_4 Standard

Standard:	Add:	Into:
10 ng/mL	100 µL of 100 ng/mL stock std. prepared in Step 4.	900 µL 1x Tris Buffer
3.33 ng/mL	250 µL of the 10 ng/mL std.	500 µL 1x Tris Buffer
1.11 ng/mL	250 µL of the 3.33 ng/mL std.	500 µL 1x Tris Buffer
0.37 ng/mL	250 µL of the 1.11 ng/mL std.	500 µL 1x Tris Buffer
0.12 ng/mL	250 µL of the 0.37 ng/mL std.	500 µL 1x Tris Buffer
0.04 ng/mL	250 µL of the 0.12 ng/mL std.	500 µL 1x Tris Buffer
0.014 ng/mL	250 µL of the 0.04 ng/mL std.	500 µL 1x Tris Buffer
0.0046 ng/mL	250 µL of the 0.014 ng/mL std.	500 µL 1x Tris Buffer

Diluted standards may be stored at 2 to 8ºC for up to 24 hours.

5. **Leukotriene E_4 Alkaline Phosphatase Tracer.**
   Reconstitute the *LTE_4 Alkaline Phosphatase Tracer* with 6 mL of 1x Tris buffer. Vortex to mix. Store this reconstituted tracer at 2 to 8ºC and use within four weeks.

6. **Leukotriene E_4 Antiserum.**
   Reconstitute the *LTE_4 Antiserum* with 6 mL of 1x Tris Buffer. Vortex to mix. Store this reconstituted antiserum at 2 to 8ºC and use within four weeks.

ASSAY METHOD: PROCEDURE

(Note: All reagents should be allowed to warm to room temperature before use.)

Figure 1: Suggested Plate Plan.

1. Pipette 150 µL 1x Tris Buffer into non-specific binding (NSB) wells, and 100 µL 1x Tris Buffer into zero standard (B_0) wells. If tissue culture medium was used to dilute the standard, substitute 100 µL of this same medium for the Tris Buffer in the NSB and B_0 wells.

2. Pipette 100 µL of *Leukotriene E_4 Standard* into the appropriate wells.
3. Pipette 100 µL of samples into the appropriate wells. Each sample should be assayed in duplicate or triplicate.
4. Pipette 50 µL of *Leukotriene E_4 Alkaline Phosphatase Tracer* into each well except the blank wells and total activity (TA) wells.
5. Pipette 50 µL of *Leukotriene E_4 Antiserum* into each well except the blank wells, TA wells and NSB wells

Note: The table below specifies reagents required for designated wells. Reading across the table, confirm you added the correct reagent(s) to each well.

Well	Tris Buffer	Std/Sample	Tracer	Antiserum
Blank	-	-	-	-
TA	-	-	5 µL (at development)	-
NSB	150 µL	-	50 µL	-
B_0	100 µL	-	50 µL	50 µL
Std/Sample	-	100 µL	50 µL	50 µL

DEFINITIONS OF KEY TERMS

Blank - No sample; to measure background absorbance.
TA (Total Activity) - total activity of the AP-linked tracer.

NSB (Non-Specific Binding) - non-immunological binding of the tracer to the well.
B_0 (Maximum Binding) - maximum amount of the tracer that the antiserum can bind in the absence of the analyte.

6. Cover each plate with a plate cover and incubate for two hours at room temperature on an orbital shaker.

7. Dissolve 5 *p*NPP tablets in 25 mL 1x DEA buffer (25 mL is sufficient to develop 100 wells). *[**Note:** Reconstituted pNPP is not stable, so we recommend that you make only the amount that you need at any one time.]*

8. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 5 times. See **DIRECTIONS FOR WASHING**.

9. Add 200 µL *p*NPP solution to each well including blank and TA wells. Add 5 µL of tracer to the TA wells. Cover the plate and allow to develop in the dark on an orbital shaker. This assay typically develops in approximately 60 to 90 minutes.

10. Wipe the bottom of the plate with a paper towel to remove any finger prints, smudges or dirt which may interfere with obtaining an accurate absorbance reading. Remove the plate cover, and read the plate at a wavelength of between 405 and 420 nm.

DATA ANALYSIS

Most plate readers come with data reduction software that plots data automatically. Alternatively a spreadsheet program can be used. The data should be plotted as $\%B/B_0$ versus log concentration of standard using either a 4-parameter logistic or a log-logit curve fit.

1. **Prepare the Data.**
   The following procedure is recommended to prepare the data prior to graphing:
   a. Subtract the absorbance of the blank wells from all wells on the plate, if not already done.
   b. Average the absorbance readings from the NSB wells.
   c. Average the absorbance readings from the B_0 wells.
   d. Subtract the average NSB from the average B_0. This is the corrected B_0.

$$\textbf{Corrected } B_0 = \textbf{Average } B_0 - \textbf{Average NSB}$$

   e. Calculate the $\%B/B_0$ for each standard and sample. To do this, subtract the average NSB absorbance from the standard and sample absorbances and divide by the corrected B_0 (from step d). Multiply by 100 to obtain $\%B/B_0$. Repeat for all wells.

$$\%B/B_0 = \left[\frac{\text{Absorbance - Average NSB}}{\text{Corrected } B_0}\right] \times 100$$

2. **Plot the Standard Curve.**
   Plot $\%B/B_0$ for all standards versus LTC_4 concentration using log (x) and linear (y) axes, and fit the data to a four parameter logistic equation. Alternatively, the data can be linearized using a logit transformation. [**Note**: Do not use $\%B/B_0$ in this calculation.]

$$\textbf{Logit } (B/B_0) = \ln [B/B_0/(1-B/B_0)]$$

   Plot the data as logit (B/B_0) *versus* log concentration of standard and perform a linear regression fit.

3. **Determine the Concentration of your Samples.**
   Calculate the $\%B/B_0$ for each sample. Determine the concentration of each sample using the equations obtained from the analysis of the standard curve. Remember to account for any dilution made to the sample prior to addition to the well. $\%B/B_0$ values of greater than 80% or less than 20% should be re-assayed as they generally fall outside of the linear range of the standard curve.

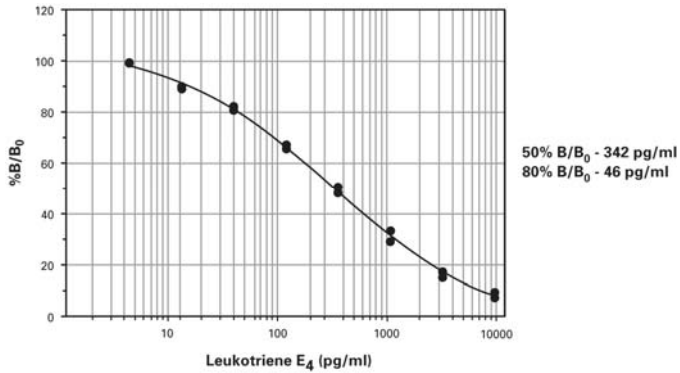
4. **Correct for Recovery (if purification was performed).**
   Divide the concentration determined in step #3 by the recovery factor. Correct for any volume changes of the sample which may have occurred during purification.

$$\text{Recovery Factor} = \frac{\text{ELISA value of spiked (pg/mL)} - \text{ELISA value of unspiked sample (pg/mL)}}{\text{Concentration of spike (pg/mL)}}$$

TYPICAL DATA

The standard curve is an example of data typically produced by this kit (Figure 2). Your results will vary from these, and it is therefore important that you run a standard curve each time you use the kit.

Figure 2: Standard curve for Leukotriene E_4



LIMITATIONS OF THE PROCEDURE

For Research Use Only. CAUTION: Not for human or animal therapeutic or diagnostic use.

PERFORMANCE CHARACTERISTICS

1. PRECISION

Intra-assay precision was determined by measuring samples containing low, medium, and high concentrations of LTE_4 multiple times in the same assay (eight samples per plate on a total of five plates). Inter-assay precision was determined by measuring low, medium, and high concentrations of the samples in eight separate assays.

	LTE_4 (pg/mL)	**Intra-assay % CV**	**Inter-assay % CV**
High	1111.1	6.4	6.3
Medium	370.4	10.3	5.0
Low	123.5	16.1	10.0

2. SPECIFICITY

Buffered solutions of a panel of substances were assayed. The following substances were tested and their corresponding cross-reactivity percentages are noted.

Analyte	**Cross-Reactivity**	**Analyte**	**Cross-Reactivity**
Leukotriene E_4	100%	Arachidonic Acid	<0.01%
Leukotriene E_5	100%	Leukotriene B_4	<0.01%
N-acetyl Leukotriene E_4	20%	Leukotriene B_5	<0.01%
Leukotriene C_4	10%	Leukotriene D_5	<0.01%
Leukotriene D_4	7%	tetranor-PGEM	<0.01%

REFERENCES

1. Shaw, A. and Krell, R.D. (1991) Peptide leukotrienes: Current status of research. *J. Med. Chem.* 34:1235-1242.
2. Mayatepek, E. and Hoffmann, G.F. (1995) Leukotrienes: Biosynthesis, metabolism, and pathophysiologic significance. *Pediatr. Res.* 37:1-9.
3. Ford-Hutchinson, A.W. (1994) Regulation of leukotriene biosynthesis. *Cancer. Metast. Rev.* 13:257-267.
4. Brink, C., Dahlen, S.-E., Drazen, J., et al. (2003) International union of pharmacology XXXVII. Nomenclature for leukotriene and lipoxin receptors. *Pharmacol. Rev.* 55:195-227.
5. Samuelsson, B. (1983) Leukotrienes: Mediators of immediate hypersensitivity reactions and inflammation. *Science* 220:568-575.
6. Lynch, K.R., O'Neill, G.P., Liu, Q., *et al.* (1999) Characterization of the human cysteinyl leukotriene CysLT 1 receptor. *Nature* 399:789-793.
7. Heise, C.E., O'Dowd, B.F., Figueroa, D.J., *et al.* (2000) Characterization of the human cysteinyl leukotriene 2 receptor. *J. Biol. Chem.* 275:30531-30536.
8. Sarau, H.M., Ames, R.S., Chambers, J., *et al.* (1999) Identification, molecular cloning, expression, and characterization of a cysteinyl leukotriene receptor. *Mol. Pharmacol.* 56:657-663.
9. Dahlén, S.-E., Björk, J., Hedqvist, P., *et al.* (1981) Leukotrienes promote plasma leakage and leukocyte adhesion in postcapillary venules: *In vivo* effects with relevance to the acute inflammatory response. *Proc. Natl. Acad. Sci. USA* 78:3887-3891.
10. Centanni, S. and Santus, P. (2002) Antileukotrienes in clinical development for asthma. *Expert Opin. Investig. Drugs* 11(1):49-58.

11. Westcott, J.Y., Johnston, K., Batt, R.A., *et al.* (1990) Measurement of peptidoleukotrienes in biological fluids. *Am. J. Physiol.* 232:2640-2648.
12. Kumlin, M., Stensvad, F., Larsson, L., *et al.* (1995) Validation and application of a new simple strategy for measurements of urinary leukotriene E4 in humans. *Clin. Exp. Allergy* 25:467-479.
13. Westcott, J.Y., Maxey, K.M., MacDonald, J., *et al.* (1998) Immunoaffinity resin for purification of urinary leukotriene E_4. *Prostaglandins and Other Lipid Mediators* 55:301-321.

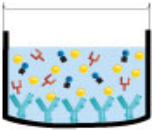
Important Licensing Information - These products may be covered by one or more Limited Use Label Licenses (see the Invitrogen Catalog or our website, www.invitrogen.com). By use of these products you accept the terms and conditions of all applicable Limited Use Label Licenses. Unless otherwise indicated, these products are for research use only and are not intended for human or animal diagnostic, therapeutic or commercial use.

Explanation of symbols

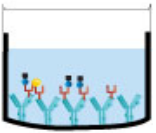
Symbol	Description	Symbol	Description
REF	Catalogue Number	LOT	Batch code
RUO	Research Use Only	IVD	*In vitro* diagnostic medical device
	Use by		Temperature limitation
	Manufacturer	EC REP	European Community authorised representative
[-]	Without, does not contain	[+]	With, contains
	Protect from light		Consult accompanying documents
	Directs the user to consult instructions for use (IFU), accompanying the product.		

Leukotriene E_4 Assay Summary

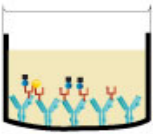
Incubate with 50 µl tracer, 50 µl antiserum, and either 50 µl standard or unknown sample, for 2 hours at RT.



Wash to remove all unbound reagents.



Add pNPP solution and incubate for 60-90 minutes.



Read Plate between 405-420 nm
Total time: 3 hours