bio-techne®

R&D SYSTEMS

# Parameter™

## $LTB_4$ Assay

Catalog Number KGE006B
SKGE006B
PKGE006B

For the quantitative determination of Leukotriene $B_4$ ($LTB_4$) concentrations in cell culture supernates, serum, and plasma.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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# INTRODUCTION

Leukotriene $B_4$ ($LTB_4$) is a potent pro-inflammatory molecule that belongs to a family of eicosanoid lipid mediators (1, 2). It is synthesized from arachidonic acid that is generated from nuclear membrane phospholipid (1, 3). In general, and upon cell activation, phospholipase A2 is directed to the nuclear membrane. Here, it generates arachidonic acid that is bound by one 18 kDa membrane-bound protein termed Five-Lipooxygenase Activating Protein (FLAP). FLAP-bound arachidonic acid is subjected to two distinct, sequential actions by 80 kDa 5-lipooxygenase (5-LO). The first 5-LO action generates 5(S)-HPETE/5-HPETE, an oxygenated form of arachidonic acid. The newly generated 5-HPETE is then acted on again by 5-LO to generate $LTA_4$ (1-4). The $LTB_4$ synthetic pathway has two key checkpoints. The first is at the level of arachidonic acid formation, where COX enzymes offer an alternative to 5-LO in the generation of prostaglandins. The second is at the level of $LTA_4$, where 69 kDa $LTA_4$ hydrolase activity will generate $LTB_4$ (a pro-inflammatory molecule), and 18 kDa $LTC_4$ synthetase activity will generate LTC/D/E4 (smooth muscle contractants) (4, 5). The key enzyme in $LTB_4$ synthesis is 5-LO, which has limited cell expression. Cells known to express 5-LO include B cells, macrophages, monocytes, mast cells, neutrophils, neurons, eosinophils, and dendritic cells (1, 2). Notably, all cells are potential sources for $LTB_4$ production. This is due to the fact that $LTA_4$ hydrolase is ubiquitously expressed, and excess $LTA_4$ generated by 5-LO expressing cells can diffuse into neighboring $LTA_4$ hydrolase-containing cells for conversion into $LTB_4$ (5).

$LTB_4$ has two known membrane receptors and one intracellular receptor. The membrane receptors are named BLT1 and BLT2 (2, 6). Both are 7-transmembrane receptors that induce $Ca^{2+}$ flux when activated. BLT1 is a high-affinity receptor for $LTB_4$ expressed by hematopoietic cells and keratinocytes, while BLT2 is a low-affinity receptor that is widely expressed (6, 7). The intracellular receptor is PPAR-γ, which becomes active upon binding to $LTB_4$. This activation promotes the expression of $LTB_4$ degradative enzymes, creating a negative feedback loop for $LTB_4$ production (1, 8).

$LTB_4$ functions are considered pro-inflammatory but vary depending upon the target cell. On hematopoietic stem cells, $LTB_4$ may induce differentiation into CFU-GEMM and CFU-GM progenitor cells (9). B cells are induced to differentiate by $LTB_4$, and in the presence of IL-4, to release IgE (2). $LTB_4$ is also considered essential for Th2-type responses. It drives IL-4 and IL-5 production, and promotes $CD4^+$ cell proliferation while inhibiting $CD8^+$ cell expansion (2, 6, 10). Under the influence of $LTB_4$, endothelial cells bind and promote neutrophil transmigration (1, 6). $LTB_4$ is perhaps best known for its activity on neutrophils. It promotes polymorphonuclear (PMN) cell migration, blocks PMN apoptosis, and induces neutrophil granule release in conjunction with reactive oxygen species generation (2, 6). Finally, it activates macrophage phagocytosis and drives mononuclear pro-inflammatory cytokine release (2, 6).

The Parameter™ $LTB_4$ Assay is a 4.5 hour forward sequential competitive enzyme immunoassay designed to measure $LTB_4$ in cell culture supernates, serum, and plasma.

## PRINCIPLE OF THE ASSAY

This assay is based on the forward sequential competitive binding technique in which $LTB_4$ present in a sample competes with a fixed amount of horseradish peroxidase (HRP)-labeled $LTB_4$ for sites on a chicken polyclonal antibody. During the incubations, the chicken polyclonal antibody becomes bound to the rabbit anti-chicken antibody coated onto the microplate. Following a wash to remove excess conjugate and unbound sample, a substrate solution is added to the wells to determine the bound enzyme activity. Following color development, the assay is stopped, and the absorbance is read at 450 nm. The intensity of the color is inversely proportional to the concentration of $LTB_4$ in the sample.

## LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples with calibrator diluent and repeat the assay.
- Any variation in diluent, operator, pipetting technique, incubation time or temperature, and kit age can cause assay variability.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in this assay, the possibility of interference cannot be excluded.

## TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- Pre-rinse the pipette tips when pipetting.
- Pipette standards and samples to the bottom of the wells. Add all other reagents to the side of the wells to avoid contamination.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution. Gently tap the side of the plate to ensure thorough mixing.
- **Samples containing chicken IgY may interfere with this assay.**

## PRECAUTIONS

Care should be taken when handling the $LTB_4$ Standard because of the known and unknown effects of eicosanoids.

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the SDS on our website prior to use.

## MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at ≤ -20 °C in a manual defrost freezer.
Do not use past kit expiration date.

| PART | PART # | CATALOG # KGE006B | CATALOG # SKGE006B | DESCRIPTION | STORAGE OF OPENED/ RECONSTITUTED MATERIAL |
|---|---|---|---|---|---|
| **$LTB_4$ Microplate** | 893074 | 1 plate | 6 plates | 96 well polystyrene microplate (12 strips of 8 wells) coated with a rabbit anti-chicken polyclonal antibody. | Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.* |
| **$LTB_4$ Conjugate** | 893641 | 1 vial | 6 vials | 6 mL/vial of $LTB_4$ conjugated to horseradish peroxidase with red dye and preservatives. | Aliquot and store for up to 1 month at ≤ -20 °C in a manual defrost freezer.* Avoid repeated freeze-thaw cycles. |
| **$LTB_4$ Standard** | 893077 | 1 vial | 6 vials | $LTB_4$ in a buffer with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.* | |
| **Primary Antibody Solution** | 893642 | 1 vial | 6 vials | 6 mL/vial of a chicken polyclonal antibody to $LTB_4$ in a buffer with blue dye and preservatives. | May be stored for up to 1 month at 2-8 °C.* |
| **Calibrator Diluent RD5-52** | 895925 | 1 vial | 6 vials | 15 mL/vial of a buffered protein base with preservatives. | |
| **Wash Buffer Concentrate** | 895209 | 2 vials | 12 vials | 11 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.* | |
| **Color Reagent A** | 895000 | 1 vial | 6 vials | 12 mL/vial of stabilized hydrogen peroxide. | |
| **Color Reagent B** | 895001 | 1 vial | 6 vials | 12 mL/vial of stabilized chromogen (tetramethylbenzidine). | |
| **Stop Solution** | 895032 | 2 vials | 12 vials | 6 mL/vial of 2N sulfuric acid. | |
| **Plate Sealers** | N/A | 4 strips | 24 strips | Adhesive strips. | |

* Provided this is within the expiration date of the kit.

KGE006B contains sufficient materials to run an ELISA on one 96 well plate.
SKGE006B (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PKGE006B). Refer to the PharmPak Contents section for specific vial counts.

## PHARMPAK CONTENTS

Each PharmPak contains reagents sufficient for the assay of 50 microplates (96 wells/plate). The package inserts supplied are the same as those supplied in the single kit packs and because of this, a few minor differences related to the number of reagents and their container sizes should be noted.

- Sufficient material is supplied to perform at least 50 standard curves; reuse of each vial may be required. The number of vials, and the number of standard curves obtained per vial will vary with the analyte.
- Wash Buffer 25X Concentrate is bulk packed in 125 mL bottles containing 100 mL.
  **Note:** *Additional wash buffer is available for purchase (R&D Systems®, Catalog # WA126).*

The reagents provided in this PharmPak are detailed below.

| PART | PART # | QUANTITY |
|---|---|---|
| **$LTB_4$ Microplate** | 893074 | 50 plates |
| **$LTB_4$ Conjugate** | 893641 | 50 vials |
| **$LTB_4$ Standard*** | 893077 | 50 vials |
| **Primary Antibody Solution** | 893642 | 50 vials |
| **Calibrator Diluent RD5-52** | 895925 | 50 vials |
| **Wash Buffer Concentrate** | 895126 | 9 bottles |
| **Color Reagent A** | 895000 | 50 vials |
| **Color Reagent B** | 895001 | 50 vials |
| **Stop Solution** | 895032 | 100 vials |
| **Plate Sealers** | N/A | 100 sheets |
| **Package Inserts** | 751936 | 2 booklets |

**If additional standard vials are needed, contact Technical Service at techsupport@bio-techne.com*

## OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 or 570 nm
- Pipettes and pipette tips
- Deionized or distilled water
- Squirt bottle, manifold dispenser, or automated microplate washer
- 500 mL graduated cylinder
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm
- Test tubes for dilution of standards
- $LTB_4$ Controls (optional; R&D Systems®, Catalog # QC116)

## SAMPLE COLLECTION & STORAGE

**The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.**

**Samples containing chicken IgY may interfere with this assay.**

**Cell Culture Supernates** - Remove particulates by centrifugation. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

**Note:** *Animal serum used in the preparation of cell culture media may contain high levels of $LTB_4$. For best results, do not use animal serum for growth of cell cultures when assaying for $LTB_4$ production. If animal serum is used as a supplement in the media, the appropriate media control should be prepared and tested in the immunoassay to determine the baseline concentration of $LTB_4$.*

**Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles to minimize the conversion of $LTA_4$ to $LTB_4$ (refer to the note on page 11).

**Plasma** - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles to minimize the conversion of $LTA_4$ to $LTB_4$ (refer to the note on page 11).

**Note:** *Citrate plasma has not been validated for use in this assay.*
*Do not use lipemic samples.*

## REAGENT PREPARATION

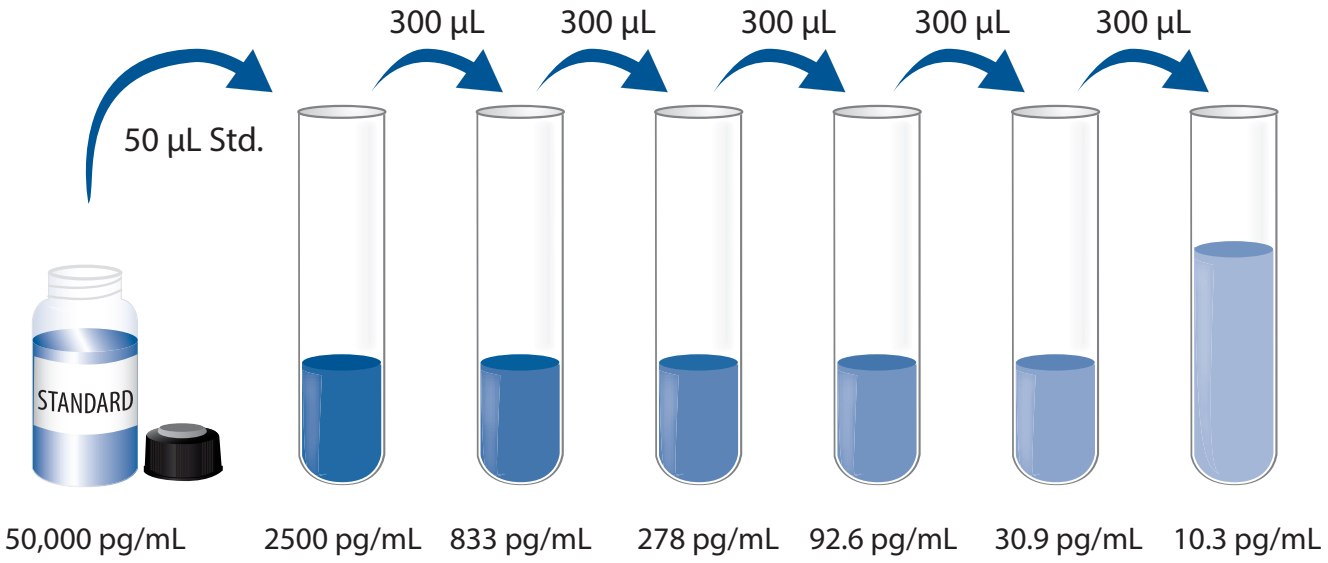
**Bring all reagents to room temperature before use.**

**Wash Buffer** - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

**Substrate Solution** - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

**$LTB_4$ Standard** - **Refer to the vial label for reconstitution volume.** Reconstitute the $LTB_4$ Standard with deionized or distilled water. This reconstitution produces a stock solution of 50,000 pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 950 µL of Calibrator Diluent RD5-52 into the 2500 pg/mL tube. Pipette 600 µL into the remaining tubes. Use the 50,000 pg/mL standard stock to produce a dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 2500 pg/mL standard serves as the high standard. Calibrator Diluent RD5-52 serves as the zero standard ($B_0$) (0 pg/mL). **Use diluted standards within 60 minutes of preparation.**



## ASSAY PROCEDURE

**Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.**

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 µL of Calibrator Diluent RD5-52 to the non-specific binding (NSB) wells.
4. Add 50 µL of Calibrator Diluent RD5-52 to the zero standard ($B_0$) wells.
5. Add 50 µL of standard, control, or sample to the remaining wells. A plate layout is provided to record the standards and samples assayed.
6. Add 50 µL of the Primary Antibody Solution to each well **(excluding the NSB wells).** All wells, except the NSBs, will now be blue in color. Cover with the adhesive strip provided. Incubate for 1 hour at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.
7. **Do not wash the plate.** Add 50 µL of $LTB_4$ Conjugate to each well. All wells will now be violet in color except the NSB wells, which will be pink. Cover with a new adhesive strip. Incubate for 3 hours at room temperature on the shaker.
8. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
9. Add 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
10. Add 100 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
11. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

## CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample then subtract the average NSB optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on a linear y-axis against the concentration on a logarithmic x-axis and draw the best fit curve through the points on the graph. Do not include the $B_0$ in the standard curve.

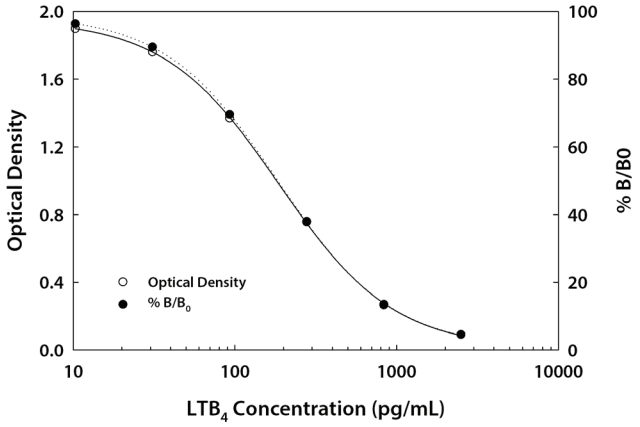
If desired, % $B/B_0$ can be calculated by dividing the corrected O.D. for each standard or sample by the corrected $B_0$ O.D. and multiplying by 100.

Calculate the concentration of $LTB_4$ corresponding to the mean absorbance from the standard curve.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

## TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.




| (pg/mL) | O.D. | Average | Corrected | % $B/B_0$ |
|---|---|---|---|---|
| NSB | 0.009<br>0.010 | 0.009 | — | — |
| 0 ($B_0$) | 1.974<br>1.980 | 1.977 | 1.968 | — |
| 10.3 | 1.908<br>1.909 | 1.908 | 1.899 | 96.4 |
| 30.9 | 1.747<br>1.794 | 1.770 | 1.761 | 89.5 |
| 92.6 | 1.356<br>1.403 | 1.379 | 1.370 | 69.6 |
| 278 | 0.756<br>0.777 | 0.766 | 0.757 | 38.0 |
| 833 | 0.269<br>0.282 | 0.275 | 0.266 | 13.5 |
| 2,500 | 0.098<br>0.102 | 0.100 | 0.091 | 4.6 |

## PRECISION

**Intra-Assay Precision** (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

**Inter-Assay Precision** (Precision between assays)
Three samples of known concentration were tested in forty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

| | Intra-Assay Precision | | | Inter-Assay Precision | | |
|---|---|---|---|---|---|---|
| **Sample** | 1 | 2 | 3 | 1 | 2 | 3 |
| **n** | 20 | 20 | 20 | 40 | 40 | 40 |
| **Mean (pg/mL)** | 170 | 481 | 847 | 164 | 516 | 879 |
| **Standard deviation** | 10 | 19 | 34 | 14.1 | 36.6 | 73.6 |
| **CV (%)** | 5.8 | 4.0 | 4.0 | 8.6 | 7.1 | 8.4 |

## RECOVERY

The recovery of $LTB_4$ spiked to levels throughout the range of the assay in various matrices was evaluated.

| Sample Type | Average % Recovery | Range |
|---|---|---|
| Cell culture media (n=4) | 95 | 86-114% |
| Serum (n=4) | 98 | 82-118% |
| EDTA plasma (n=4) | 91 | 82-112% |
| Heparin plasma (n=4) | 98 | 82-115% |

## LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of $LTB_4$ were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

| | | Cell culture media (n=4) | Serum (n=4) | EDTA plasma (n=4) | Heparin plasma (n=4) |
|---|---|---|---|---|---|
| 1:2 | Average % of Expected | 101 | 89 | 97 | 95 |
| | Range (%) | 93-107 | 79-100 | 88-102 | 89-103 |
| 1:4 | Average % of Expected | 105 | 102 | 113 | 108 |
| | Range (%) | 98-110 | 97-108 | 108-120 | 104-116 |
| 1:8 | Average % of Expected | 108 | 101 | 110 | 102 |
| | Range (%) | 101-115 | 91-118 | 99-120 | 95-111 |
| 1:16 | Average % of Expected | 113 | 102 | 108 | 102 |
| | Range (%) | 101-124 | 99-106 | 100-117 | 96-106 |

## SENSITIVITY

Fifty assays were evaluated and the minimum detectable dose (MDD) of $LTB_4$ ranged from 3.7-10.9 pg/mL. The mean MDD was 8.2 pg/mL.

The MDD was determined by subtracting two standard deviations from the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

## SAMPLE VALUES

**Serum/Plasma** - Samples from apparently healthy volunteers were evaluated for the presence of $LTB_4$ in this assay. No medical histories were available for the donors used in this study.

| Sample Type | Mean of Detectable (pg/mL) | % Detectable | Range (pg/mL) |
|---|---|---|---|
| Serum (n=35) | 138 | 100 | 33.5-564 |
| EDTA plasma (n=35) | 38.2 | 97 | ND-72.8 |
| Heparin plasma (n=35) | 54.3 | 100 | 11.5-129 |

ND=Non-detectable

**Note:** *$LTA_4$ hydrolase, a metal dependent enzyme present in serum and plasma, converts $LTA_4$ to $LTB_4$ (11). In samples collected in the presence of EDTA, this conversion is inhibited at the time the blood is drawn resulting in a more accurate measurement of circulating $LTB_4$. Other samples, especially serum samples, typically have higher levels of $LTB_4$ that result from the conversion of $LTA_4$ after the sample is drawn.*

**Cell Culture Supernates** - Human peripheral blood cells (1 x $10^6$ cells/mL) were cultured in RPMI supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10 µg/mL PHA. Aliquots of the cell culture supernates were removed and assayed for levels of $LTB_4$.

| Condition | Day 1 (pg/mL) | Day 6 (pg/mL) |
|---|---|---|
| Stimulated | 222 | 96.8 |
| Unstimulated | 61.8 | 56.6 |

ND=Non-detectable

**Note:** *Samples values have not been corrected for $LTB_4$ present in media.*

## SPECIFICITY

The factors listed below were prepared at 50 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range $LTB_4$ control were assayed for interference. No significant cross-reactivity or interference was observed.

### Factors:

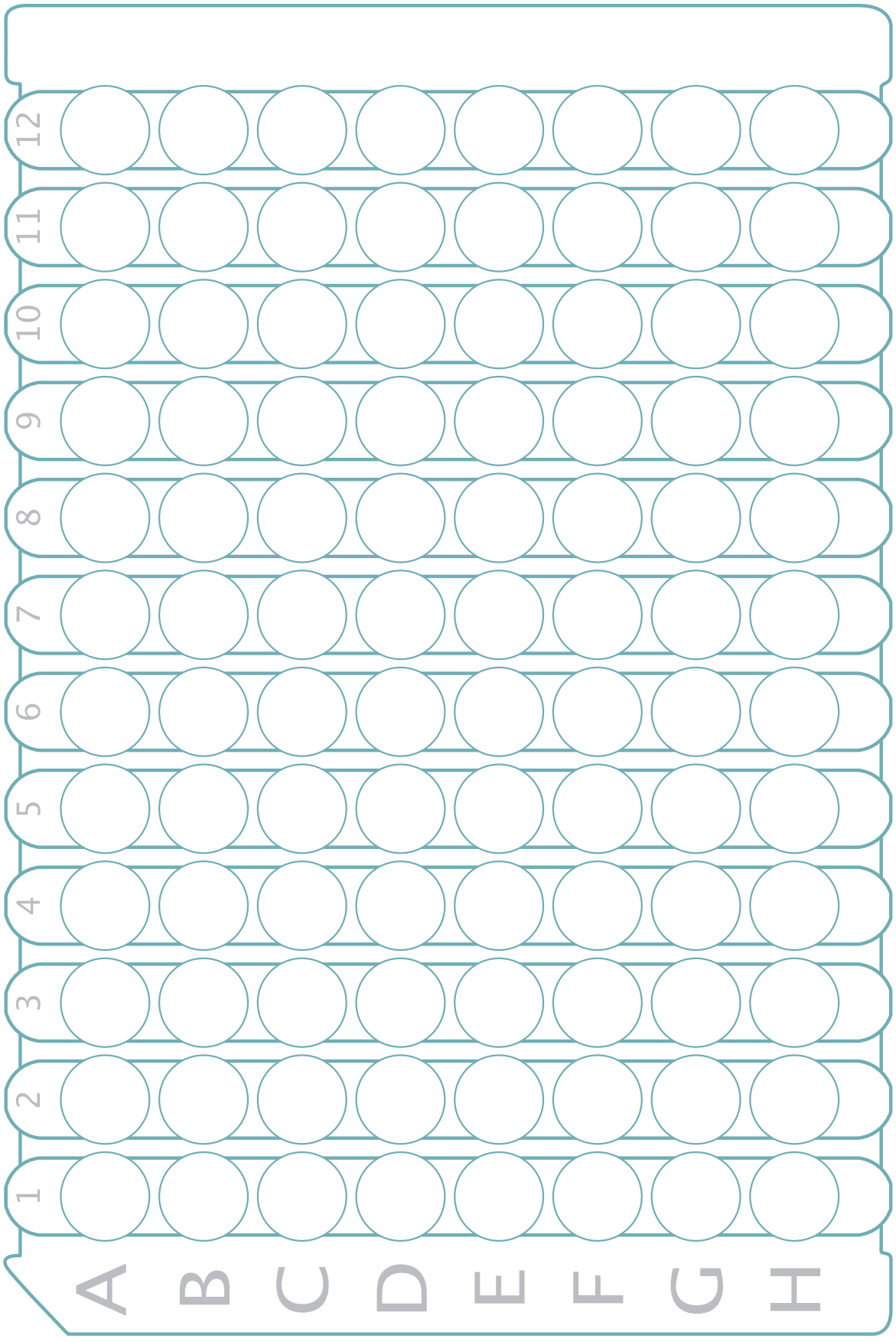
5(S)-HETE
12(S)-HETE
15(S)-HETE
Arachidonic acid
Arachidonyl ethanolamide
$LTC_4$
$LTD_4$

## REFERENCES


1. Yokomizo, T. *et al.* (2001) Arch. Biochem. Biophys. **385**:231.
2. Hedi, H. and G. Norbert (2004) J. Biomed. Biotechnol. **4**:99.
3. Radmark, O. *et al.* (2007) Trends Biochem. Sci. **32**:332.
4. Haeggstrom, J.Z. and A. Wetterholm (2002) Cell. Mol. Life Sci. **59**:742.
5. Werz, O. (2002) Curr. Drug Targets Inflamm. Allergy **1**:23.
6. Tager, A.M. and A.D. Luster (2003) Prostaglandins Leukot. Essent. Fatty Acids **69**:123.
7. Iizuka, Y. *et al.* (2005) J. Biol. Chem. **280**:24816.
8. Kikuta, Y. *et al.* (2002) Prostaglandins Other Lipid Mediat. **68-69**:345.
9. Chung, J.W. *et al.* (2005) Exp. Mol. Med. **37**:45.
10. Terawaki, K. *et al.* (2005) J. Immunol. **175**:4217.
11. Fitzpatrick, F. *et al.* (1983) Proc. Natl. Acad. Sci. USA **80**:5425.

## PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



## NOTES