GeneBLAzer® LTB4R-Ga15-NFAT-*bla* CHO-K1 Cells

Catalog Numbers – K1748

Cell Line Descriptions

GeneBLAzer ® LTB4R-Ga15-NFAT-*bla* CHO-K1 cells contain the human Leukotriene B4 Receptor (LTB4R), (Accession # NM_181657) stably integrated into the GeneBLAzer® Ga15-NFAT-bla CHO-K1 cell line. GeneBLAzer® Ga15-NFAT-bla CHO-K1 cells (Cat. no. K1537) contain a beta-lactamase (*bla*) reporter gene under control of the NFAT Response Element.

DA cells are irreversibly division arrested using a low-dose treatment of Mitomycin-C, and have no apparent toxicity or change in cellular signal transduction. Both GeneBLAzer ® LTB4R-Ga15 CHO-K1 DA cells and GeneBLAzer ® LTB4R-Ga15-NFAT-*bla* CHO-K1 cells are functionally validated for Z'-factor and EC_{50} concentrations of LTB4 (Figure 1). In addition, GeneBLAzer ® LTB4R-Ga15-NFAT-*bla* CHO-K1 cells have been tested for assay performance under variable conditions.

Target Description

Leukotriene B4 (LTB4) is a lipid mediator which activates leukocytes and is involved in host defenses and inflammation. LTB4R (BLT1), a high affinity receptor for LTB4, is expressed in inflammatory cells (and to a lesser degree in spleen and thymus) and is inducible in macrophages upon activation. Therefore, understanding the mechanisms of LTB4R expression and activation is important for the development of potential anti-inflammatory treatments.

LTB4 has been shown to be related to inflammatory diseases such as rheumatoid arthritis, bronchial asthma, psoriasis, ulcerative colitis, and postischemic tissue injuries. Additional studies have hinted at a role for LTB4R in atherosclerotic plaque formation and vascular disease.

Validation Summary

Testing and validation of this assay was evaluated in a 384-well format using LiveBLAzer™-FRET B/G Substrate.

1. **LTB4 dose response under optimized conditions**

	Dividing Cells
EC_{50}	62 nM
Z'-factor	0.88
Recommended cell no. /well	= 10,000
Recommended Stim. Time	= 4 hrs
Max. [Stimulation]	= 1000 nM

2. **Agonist 2nd messenger dose response**

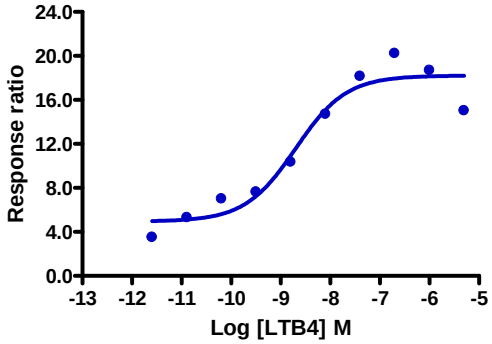
LTB4 EC_{50}	= 183 nM

3. **Assay performance with variable cell number.**

4. **Assay performance with variable stimulation time.**

Primary Agonist Dose Response

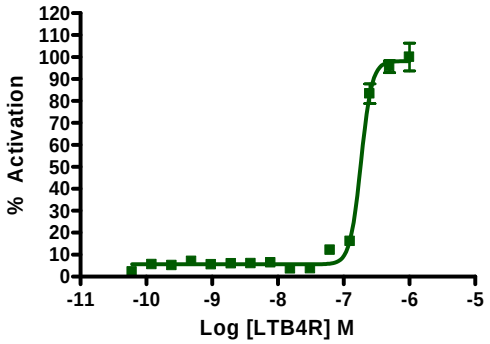
Figure 1 — GeneBLAzer® LTB4R-Ga15 CHO-K1 DA and LTB4R-Ga15-NFAT-*bla* CHO-K1 cells dose response to LTB4 under optimized conditions



GeneBLAzer® LTB4R-Ga15 CHO-K1 DA cells and GeneBLAzer® LTB4R-Ga15-NFAT-*bla* CHO-K1 cells (10,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours. Cells were stimulated with a dilution series of LTB4 (Cayman Chemical 20110) in the presence of 0.1% DMSO for 4 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate for 2 hours. Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and % Activation plotted for each replicate against the concentrations of LTB4.

2nd Messenger Dose Response

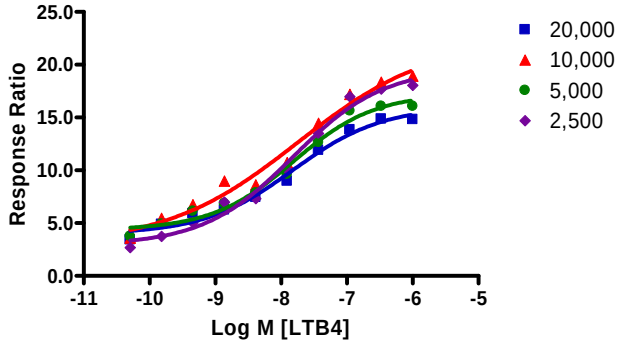
Figure 4 — GeneBLAzer® LTB4R-Ga15-NFAT-*bla* CHO-K1 2nd messenger dose response to LTB4 under optimized conditions.



GeneBLAzer® LTB4R-Ga15-NFAT-*bla* CHO-K1 cells were loaded with Fluo4-AM and tested for a response to LTB4.

Assay Performance with Variable Cell Number

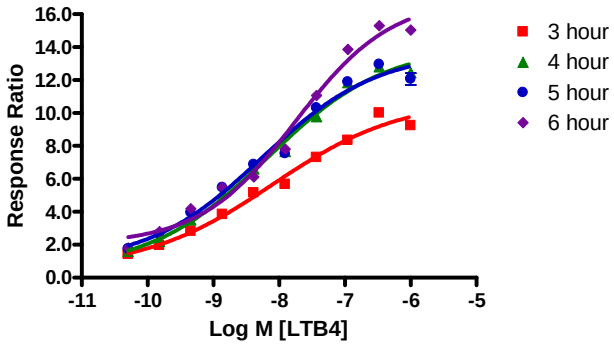
Figure5 — GeneBLAzer® LTB4R-Ga15-NFAT-*bla* CHO-K1 cells dose response to LTB4with 10K or 15K cells/ well



GeneBLAzer® LTB4R-Ga15-NFAT-*bla* CHO-K1 cells were plated in a 384-well format at 2,500, 5,000, 10,000 or 15,000 cells/well and incubated for 16-24 hours. On the day of the assay, cells were stimulated with LTB4 (Cayman Chemical, cat# 20110) in the presence of 0.1% DMSO for 4 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate for 2 hours. Fluorescence emission values at 460 nm and 530 nm for the various cell numbers were obtained using a standard fluorescence plate reader and the Response Ratios plotted against the indicated concentrations of LTB4.

Assay Performance with Variable Stimulation Time

Figure 6 – GeneBLAzer® LTB4R-Ga15-NFAT-*bla* CHO-K1 cells dose response to LTB4 with 3, 4 and 5 hour stimulation times



GeneBLAzer® LTB4R-Ga15-NFAT-*bla* CHO-K1 cells (10,000 cells/well) were plated the day before the assay in a 384-well assay plate. LTB4 (Cayman Chemical, cat# 20110) was then added to the plate over the indicated concentration range for 3, 4, 5, or 6 hrs in 0.1% DMSO. The cells were then loaded for 2 hours with LiveBLAzer™-FRET B/G Substrate. Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and the Response Ratios plotted against the indicated concentrations of LTB4.