GeneBLAzer® FPRL-1 CHO-K1 DA Assay Kit

GeneBLAzer® FPRL-1 Gα15-NFAT-*bla* CHO-K1 Cells

Catalog Numbers – K1357 and K1532

Cell Line Descriptions

GeneBLAzer® FPRL-1-Gα15 CHO-K1 DA (Division Arrested) cells and GeneBLAzer® FPRL-1-Gα15-NFAT-*bla* CHO-K1 cells contain the human Formyl Peptide-like 1 receptor (FPRL-1), (Accession #NM_001462) stably integrated into the GeneBLAzer® Gα15-NFAT- *bla* CHO-K1 cell line. GeneBLAzer®Gα15-NFAT-*bla* CHO-K1 cells (Cat. no. K1537) contain a beta-lactamase (*bla*) reporter gene under control of the Nuclear Factor of Activated T-cells (NFAT) response element, in addition to the promiscuous G-protein, Gα15. Division Arrested (DA) cells are available as an Assay Kit,which includes cells and sufficient substrate to analyze 1 x 384-well plate.

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® FPRL-1-Gα15 CHO-K1 DA cells and GeneBLAzer® FPRL-1-Gα15-NFAT-*bla* CHO-K1 cells are functionally validated for Z'-factor and EC_{50} concentrations of WKYMV(M); (Figure 1). In addition, GeneBLAzer® FPRL-1-NFAT-*bla* CHO-K1 cells have been tested for assay performance under variable conditions, including DMSO concentration, cell number, stimulation time, and substrate loading time. Additional testing data using alternate stimuli are also included.

Target Description

FPRL-1 has many functions in the body including airway hyper-responsiveness, pulmonary inflammation (1), amyloidgenic disease and gastroduodenol diseases. In a separate mouse model for ear inflammation, topical application of LXA_4 to mouse ears inhibited neutrophil infiltration thus reducing inflammation (2). These results suggest that FPRL-1 may be involved in anti-inflammatory responses.

FPRL-1 has also been reported to play a potential role in amyloidogenic diseases as three polypeptides associated with these diseases are specific chemotactic agonists for FPRL-1. These polypeptides are serum amyloid A (SAA) (3), a peptide fragment of the human prion protein (4), and a 42 amino acid form of β amyloid peptide ($A\beta_{42}$) (5,6). SAA is an acute phase protein that can lead to progressive loss of organ function (7). A 21 amino-acid fragment of the aberrant human prion protein can form fibrils *in vitro* and elicits an array of inflammatory responses by mononuclear phagocytes (8). This prion-peptide fragment has been shown to activate FPRL-1, induce monocyte migration, and increase the production of the proinflammatory cytokines, TNF-α and IL-1β (4). Therefore, FPRL-1 may also participate in the inflammatory pathology seen in prion disease. Additionally FPRL-1 has been shown to be activated by $A\beta_{42}$ (5, 6). $A\beta_{42}$ is a fragment of the amyloid precursor protein and is a major component of the senile plaques found in brain tissue of patients suffering from Alzheimer's Disease (9). FPRL-1 may also contribute to the proinflammatory effects of $A\beta_{42}$ by inducing monocyte migration and activation. Brain tissues of AD patients have shown high levels of FPRL-1 mRNA in the mononuclear phagocytes surrounding and infiltrating the plaques (5).

Validation Summary

Performance of this assay was validated under optimized conditions in 384-well format using LiveBLAzer™-FRET B/G Substrate.

1. **W(M) Peptide agonist dose response under optimized conditions**

	DA cells	Dividing Cells
EC_{50}	7 nM	13 nM
Z'-factor	0.77	0.66

Optimum cell no.	= 5K cells/well
Optimum[DMSO]	= up to 1%
OptimumStim. Time	= 4 hours
Max. [Stimulation]	= 500nM

2. **Alternate agonist dose response**

W Peptide Isoform	EC_{50}	= 581pM
CKB8-1	EC_{50}	= 1.6nM

3. **Antagonist dose response**
   See antagonist dose response section

4. **Agonist Dose Response Using Fluo-4NW**
   W(M) Peptide EC_{50} = 0.1 nM

Assay Testing Summary

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

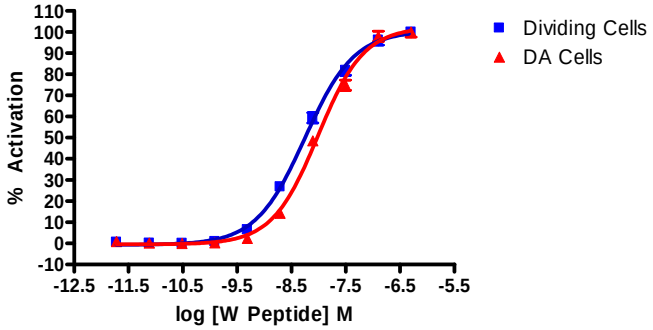
6. **Assay performance with variable stimulation time**

7. **Assay performance with variable substrate loading time**

8. **Assay performance with variable DMSO concentration**

Primary Agonist Dose Response

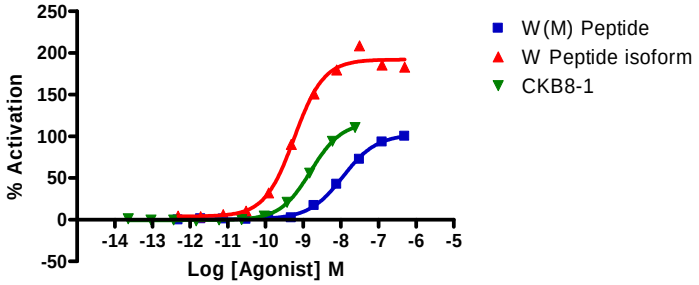
Figure 1 — GeneBLAzer® FPRL-1-Gα15 CHO-K1 DA and FPRL-1-Gα15-NFAT-*bla* CHO-K1 dose response to WKYMV(M) under optimized conditions



GeneBLAzer® FPRL-1 CHO-K1 DA cells and GeneBLAzer® FPRL-1-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 WKYMV(M) in the presence of 0.5% DMSO for 5 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 WKYMV(M) (n=6 for each data point).

Alternate Agonist Dose Response

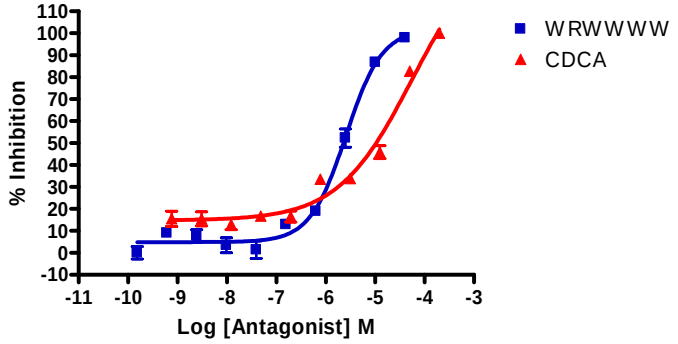
Figure 2 — GeneBLAzer® FPRL-1-Gα15-NFAT-*bla* CHO-K1 dose response to W(M) Peptide, W Peptide Isoform and CKB8-1



GeneBLAzer® FPRL-1-Gα15-NFAT-*bla* CHO-K1 cells (5,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours. Prior to assay the plating media was removed and assay media was added. Cells were stimulated with a dilution series of W(M) Peptide (Phoenix Pharma. #072-11), W Peptide Isoform (Phoenix Pharma. #072-12) or CKB8-1 (R&D Systems #508-CK-025) in the presence of 0.5% 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 the % Activation plotted against the concentrations of the agonists (n=8 for each data point).

Antagonist Dose Response

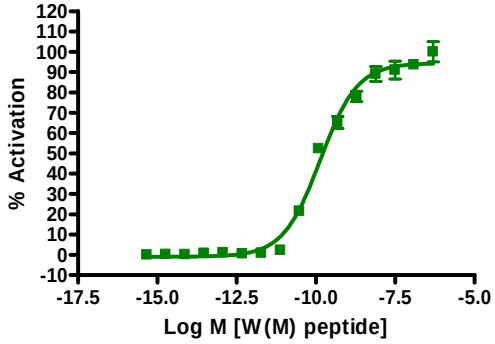
Figure 3 — GeneBLAzer® FPRL-1-Gα15-NFAT-*bla* CHO-K1 dose response to WRW^4 and CDCA



GeneBLAzer® FPRL-1-Gα15-NFAT-*bla* CHO-K1 cells (5,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours. Prior to assay the plating media was removed and assay media was added. Cells were treated with dilution series of the antagonists WRW^4 (Phoenix Pharma. #072-18), and CDCA (Sigma #C9377) and incubated at 37 degrees C for 30min., followed by 20nM W Peptide agonist (Phoenix Pharma. #072-11) stimulation for 4 hours in 0.5% DMSO. 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 % Inhibition was plotted against the concentrations of the antagonist. IC_{50}s for WRW4 and CDCA were 2.6 µM and 56µM, respectively. The data shows the correct rank order potency for these antagonists.

Agonist Dose Response Using Fluo-4NW

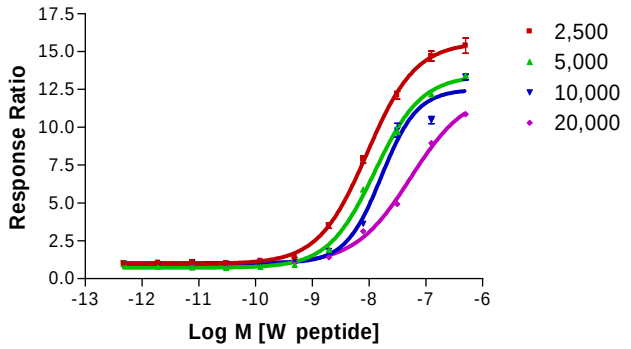
Figure 4 — GeneBLAzer® FPRL-1-Gα15-NFAT-*bla* CHO-K1 dose response to W peptide using Fluo-4NW



GeneBLAzer® FPRL-1-Gα15-NFAT-*bla* CHO-K1 cells (5,000 cells/well) plated in a 384-well format and incubated for 16-20 hours. Cells were then incubated with Fluo-4NW for 30 min. at 37°C, followed by 30 min. at room temperature. Cells were then stimulated with a dilution series of W(M) Peptide (Phoenix Pharma. #072-11) in the presence of 0.5% DMSO. Fluorescence emission values at 516 nm were obtained and the % Activation plotted against the indicated concentrations of W(M) Peptide (n=16 for each data point).

Assay Performance with Variable Cell Number

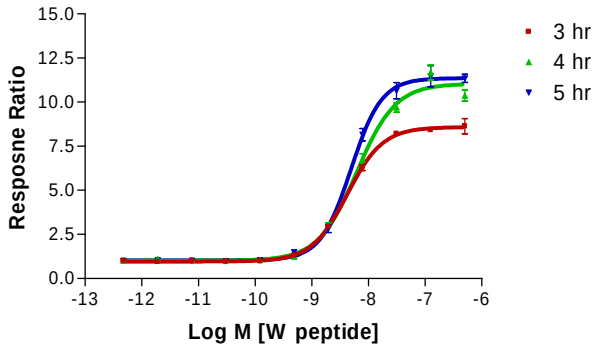
Figure 5— GeneBLAzer® FPRL-1-Gα15-NFAT-*bla* CHO-K1 dose response using 2.5, 5, 10, and 20K cells/well



GeneBLAzer® FPRL-1-Gα15-NFAT-*bla* CHO-K1 cells were plated at 2,500, 5000, 10,000 or 20,000 cells/well in a 384-well format and incubated for 16-20 hours. Prior to assay the plating media was removed and assay media added. Cells were stimulated with a dilution series of W Peptide (Phoenix Pharma. #072-11) in the presence of 0.5% 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 were plotted against the concentrations of W Peptide (n=8 for each data point).

Assay performance with Variable Stimulation Time

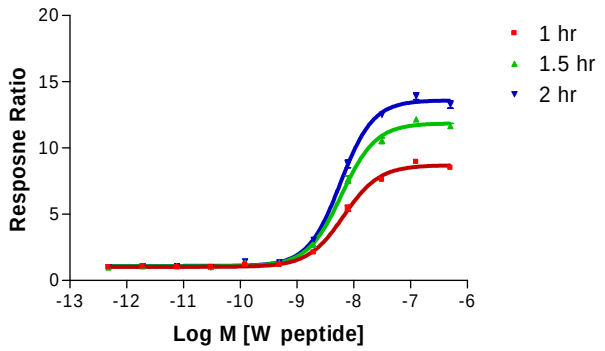
Figure 6 – GeneBLAzer® FPRL-1-Gα15-NFAT-*bla* CHO-K1 dose response using 3, 4, and 5 hour stimulation times



GeneBLAzer® FPRL-1-Gα15-NFAT-*bla* CHO-K1 cells were plated at 5,000 cells/well in a 384-well format and incubated for 16-20 hours. Prior to assay the plating media was removed and assay media was added. Cells were stimulated with a dilution series of W Peptide (Phoenix Pharma. #072-11) in the presence of 0.5% DMSO for 3, 4 or 5 hours and 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 for each substrate loading time were plotted against the concentrations of W Peptide (n=8 for each data point).

Assay performance with Variable Substrate Loading Time

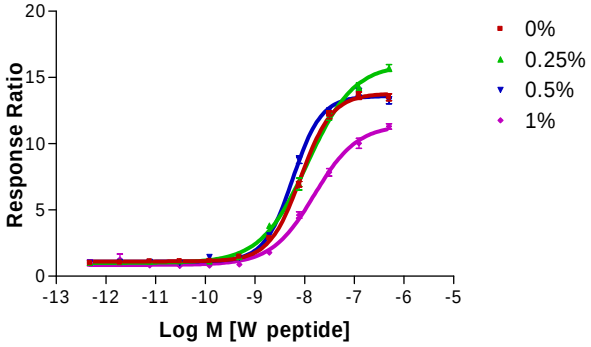
Figure 7 – GeneBLAzer® FPRL-1-Gα15-NFAT-*bla* CHO-K1 dose response using 1, 1.5, and 2 hour substrate loading times



GeneBLAzer® FPRL-1-Gα15-NFAT-*bla* CHO-K1 cells were plated at 5,000 cells/well in a 384-well format and incubated for 16-20 hours. Prior to assay the plating media was removed and assay media was added. Cells were stimulated with a dilution series of W Peptide (Phoenix Pharma. #072-11) in the presence of 0.5% DMSO for 4 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate for either 1, 1.5, or 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 were plotted against the concentrations of W Peptide (n=8 for each data point).

Assay Performance with variable DMSO concentration

Figure 8 – GeneBLAzer® FPRL-1-Gα15-NFAT-*bla* CHO-K1 dose response using 0, 0.25, 0.5 and 1% DMSO.



GeneBLAzer® FPRL-1-Gα15-NFAT-*bla* CHO-K1 cells were plated at 5,000 cells/well in a 384-well format and incubated for 16-20 hours. Prior to assay the plating media was removed and assay media was added. Cells were stimulated with a dilution series of W Peptide (Phoenix Pharma. #072-11) in the DMSO at concentrations from 0%-1%. Cells were stimulated for 4 hrs with agonist and 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 were plotted for each DMSO concentration were plotted against the concentrations of W Peptide (n=8 for each data point).

References

1) Levy, B.D., De Sanctis, G.T., Devchand, P.R., Kim, E., Ackerman, K., Schmidt, B.A., Szczeklik, W., Drazen, J.M., and Serhan, C.N. (2002) *Nature Medicine* **8,** 1018-1023.

2) Takano, T., Fiore, S., Maddox, J.F., Brady, H.R., Petasis, N.A., and Serhan, C.N. (1997) *J. Exp. Med.* **185,** 1693-1704.

3) Su, S.B., Gong, W., Gao, J.-L., Shen, W., Murphy, P.M., Oppenheim, J.J., and Wang, J.M. (1999) *J. Exp. Med.* **189,** 395-402.

4) Le, Y., Yazawa, H., Gong, W., Yu, Z., Ferrans, V.J., Murphy, P.M., and Wang, J.M. (2001) *J. Immunol.* **166,** 1448-1451.

5) Le, Y., Gong, W., Tiffany, H.L., Tumanov, A., Nedospasov, S., Shen, W., Dunlop, N.M., Gao, J.-L., Murphy, P.M., Oppenheim, J.J., and Wang, J.M. (2001) *J. Neurosci.* **21,** 1-5.

6) Yazawa, H., Yu, Z.-X., Takeda, K., Le, Y., Gong, W., Rerrans, V.J., Oppenheim, J.J., Li, C.C.H., and Wang, J.M. (2001) *FASEB* **15,** 2454-2462.

7) Stone, M.J. (1990) *Blood* **75,** 531-545.

8) Brown, D.R. and Kretzschmar, H.A. (1997) *Histol. Hisopath.* **12,** 883-892.

9) Neuroinflammatory Working Group (2000) *Neurobiol. Aging* **21,** 383-421.