Optimization of the GeneBLAzer® GLP1R CRE-bla CHO-K1 Cell Line

GeneBLAzer[®] GLP1R-CRE-*bla* CHO-K1 Cells

Catalog Numbers – K1783

Cell Line Descriptions

GeneBLAzer[®] GLP1R-CRE-*bla* CHO-K1 cells contain the human Glucagon-like Peptide 1 Receptor (GLP1R), (Accession # NM_002062) stably integrated into the CellSensor[®] CRE-bla CHO-K1 cell line. CellSensor[®] CRE-bla CHO-K1 cells (Cat. no. K1535) contain a beta-lactamase reporter gene under control of the CRE.

The GeneBLAzer[®] GLP1R-CRE-*bla* CHO-K1 cells are functionally validated for Z'-factor and EC₅₀ concentrations of GLP-1 (Figure 1). In addition, GeneBLAzer[®] GLP1R-CRE-*bla* CHO-K1 cells have been tested for assay performance under variable conditions.

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Validation Summary

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

1. GLP-1 dose response under optimized conditions

| EC ₅₀ Z'-factor | <u>Dividing Cells</u> 197 pM 0.67 |
|-------------------------------|---|
| Recommended cell no. /well | = 10,000 |
| Recommended Stim. Time | = 5 hrs |
| Max. [Stimulation] | = 10 nM |

2. Agonist 2nd messenger dose response

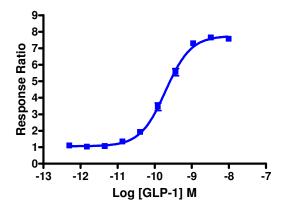
 $GLP-1 EC_{50} = 488 pM$

3. Antagonist dose response

Exendin-3 (9-39) IC₅₀= 70 nM

Primary Agonist Dose Response

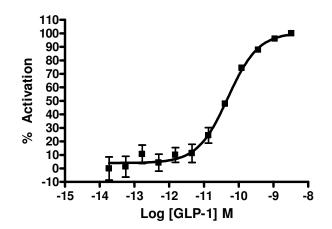
Figure 1 — GeneBLAzer $^{\otimes}$ GLP1R-CRE-*bla* CHO-K1 cells dose response to GLP-1 under optimized conditions



GeneBLAzer[®] GLP1R-CRE-*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 GLP-1 (Sigma G3265) in the presence of 0.1% 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 GLP-1.

2nd Messenger Dose Response

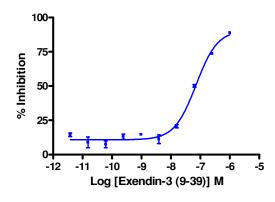
Figure 2 — GeneBLAzer $^{\rm 8}$ GLP1R-CRE-bla CHO-K1 $2^{\rm nd}$ messenger dose response to GLP-1 under optimized conditions.



GeneBLAzer[®] GLP1R-CRE-*bla* CHO-K1 cells were tested for a response to GLP-1 using a TR-FRET cAMP kit.

Antagonist Dose Response

Figure 3 — GeneBLAzer[®] GLP1R-CRE-*bla* CHO-K1 dose response to Exendin-3 (9-39)



GeneBLAzer[®] GLP1R-CRE-*bla* CHO-K1 cells (10,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours. Cells were exposed to Exendin-3 (9-39) (Tocris 2081) for 30 min. and then stimulated with an EC80 concentration of GLP-1 (Sigma G3265) in the presence of 0.1% DMSO for 5 hours. Cells were then loaded with LiveBLAzerTM-FRET B/G Substrate for 2 hours. Fluorescence emission values at 460 nm and 530 nm for the various substrate loading times were obtained using a standard fluorescence plate reader and the % Inhibition plotted against the indicated concentrations of Exendin-3 (9-39).