Validation & Assay Performance Summary

CellSensor® CRE-*bla* Jurkat Cell Line

Cat. no. K1134

This cell-based assay has been thoroughly tested and validated by Invitrogen and is suitable for immediate use in a screening application. The following information illustrates the high level of assay testing completed and the validation of assay performance under optimized conditions.

Pathway Description

Cyclic AMP (cAMP) is produced by adenyl cyclase when a Gs-coupled GPCR is activated by a ligand. cAMP activates Protein Kinase A (PKA) which then activates cAMP responsive element binding protein (CREB). CREB can bind a region of DNA called CRE(Cyclic AMP Response Element) to modulate transcriptional activity.

Cell Line Description

The CellSensor® CRE-*bla* Jurkat cell line contains a beta-lactamase reporter gene under control of the cAMP Response Element (CRE) stably integrated into Jurkat cells. This cell line is validated for EC_{50} and Z'-Factor under optimized conditions using Forskolin. This cell line has also been tested for assay performance under variable experimental conditions, including stimulation time, substrate loading time and DMSO concentration. This cell line may be used as a parental cell line in the development of Gs-coupled GPCR assays.

Validation Summary

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

1. **Primary agonist dose response under optimized conditions(n=3)**

Forskolin EC_{50}	= 9.7µM
Z'-Factor (EC_{100})	= 0.79
Response Ratio	= 6.5
Optimum cell no.	= 20K cells/well
Optimum [DMSO]	= 0.5%
Optimum Stim.Time	= 5 hours

2. **Cell culture and maintenance**
   See Cell Culture and Maintenance Section and Table 1

Assay Testing Summary

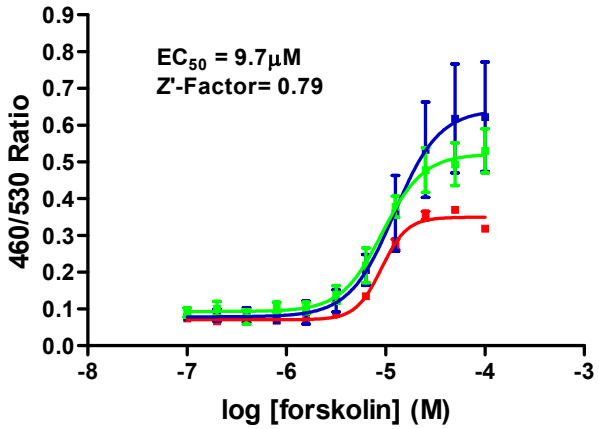
3. **Assay performance with variable stimulation time**

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

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

Primary Agonist Dose Response

Figure 1 — CRE-*bla* JURKAT dose response to Forskolin under optimized conditions



CRE-*bla* Jurkat cells (20,000 cells/well) were assayed on three separate days represented by the three curves shown on the graph. Cells were plated the day of the assay in a 384-well format and stimulated with Forskolin (Sigma #F6886) over the indicated concentration range 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 the Response Ratios plotted against the indicated concentrations of Forskolin (n=16 for each data point).

Cell Culture and Maintenance

Thaw and culture cells in Growth Medium without Blasticidin a Passage or feed cells at least twice a week and maintain them in a 37°C/5% CO_2 incubator. Maintain cells between $1x10^5$ and $2x10^6$ cells/ml. Do not allow cells to reach confluence.

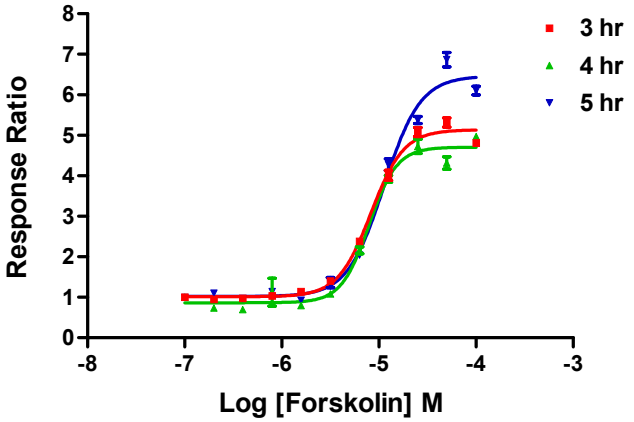
Note: We recommend passing cells for three passages after thawing before using them in the beta-lactamase assay. **Cells must be at a density greater than $2x10^6$ cells/ml on the day of the assay.** For optimal cell line performance, use dialyzed FBS (Invitrogen# 26400-036). For more detailed cell growth and maintenance directions, please refer to the protocol.

Table 1 – Cell Culture Media

Component	Growth Medium	Assay Medium	Freezing Medium
RPMI 1640	90%	90%	80%
Dialyzed FBS **DO NOT SUBSTIUTE!**	10%	10%	10%
NEAA	0.1 mM	0.1 mM	0.1 mM
Sodium Pyruvate	1 mM	1mM	1 mM
Penicillin	100 U/mL	100 U/mL	--
Streptomycin	100 µg/mL	100 µg/mL	--
DMSO	--	--	10%

Assay Performance with Variable Stimulation Time

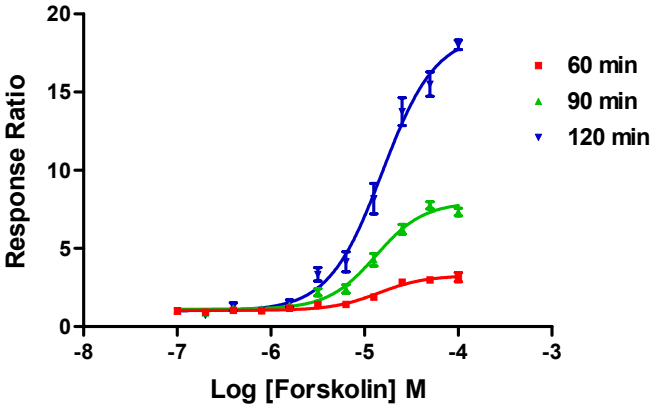
Figure 2 – CRE-*bla* Jurkat dose response to forskolin with 3, 4 and 5 hour stimulation times



CRE-*bla* Jurkat cells (20,000 cells/well) were plated the day of the assay in a 384-well assay plate. Forskolin (Sigma #F6886) was then added to the plate over the indicated concentration range. Plates were treated for 3, 4 or 5 hrs in 0.5% DMSO 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 plotted for each stimulation time against the indicated concentrations of Forskolin (n=8 for each data point).

Assay Performance with Variable Substrate Loading Time

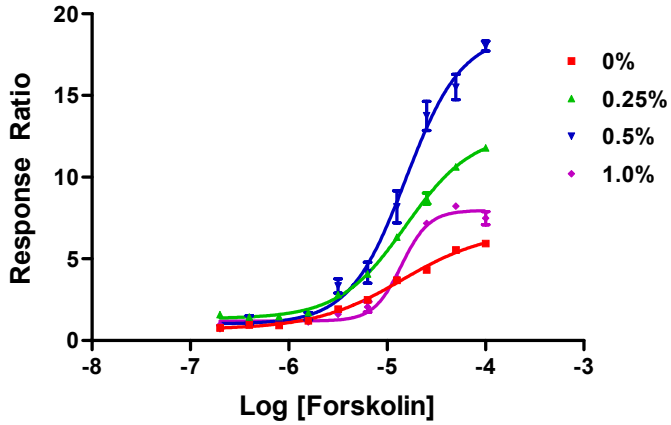
Figure 3 — CRE-*bla* Jurkat dose response to forskolin with 1, 1.5, 2, and 2.5 hour substrate loading times



CRE-*bla* JURKAT cells were plated the day of the assay at 20,000 cells/well in a 384-well format. Cells were treated with Forskolin (Sigma #F6886) over the indicated concentration range in the presence of 0.5% DMSO for 5 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 substrate loading times were obtained using a standard fluorescence plate reader and the Response Ratios plotted for each substrate loading time against the indicated concentrations of Forskolin (n=8 for each data point).

Assay Performance with Variable [DMSO]

Figure 4 – CRE-*bla* Jurkat dose response to forskolin with 0, 0.25, 0.5 and 1% DMSO



CRE-*bla* Jurkat cells (20,000 cells/well) were plated the day of the assay in a 384-well assay plate. Forskolin (Sigma #F6886) was then added to the plate over the indicated concentration range for 5 hrs with 0, 0.25, 0.5 or 1% final DMSO concentrations. 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 for each DMSO concentration were plotted against the indicated concentrations of Forskolin (n=8 for each data point).