

Validation & Assay Performance Summary



CellSensor® ESRE-*bla* HeLa Cell Line

Cat. no.

Pathway Description

Endoplasmic Reticulum (ER) stress is associated with a variety of pathophysiological conditions, such as neurodegenerative diseases, diabetes, tumor growth under hypoxic conditions, and ischemic heart disease. Proteins in the ER misfold or unfold, and accumulate under stress conditions, which promote the expression of ER stress responsive genes. One of the mechanisms for mediating ER stress response is the activation of transcription factor ATF6. The quiescent form of ATF6 (p90ATF6), a type II-transmembrane protein, is embedded in the ER membrane and proteolyzed in an ER stress-dependent manner. The liberated N-terminal fragment (p50ATF6) translocates to the nucleus, binding to ER stress response element (ERSE) present in the proximal promoter regions of many ER stress-responsive proteins including ER chaperones.

Cell Line Description

To better understand the pathological processes and provide novel avenues to potential therapies, ESRE-*bla* HeLa cells are engineered to express beta-lactamase under the control of ER stress response element. This is a clonal population isolated by FACS and its dose response curves with tunicamycin and thapsigargin are performed. This cell line also response to other known ER stress inducers.

Validation Summary

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

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

Average tunicamycin EC₅₀ = 189 nM
Average Z'-Factor (EC₁₀₀) = 0.73
Average Response Ratio = 4.8

Recommended cell no. = 5,000 cells/well
Recommended [DMSO] = up to 0.5 %
Stimulation Time = 5 hours
Max. [Stimulation] tunicamycin = 1000 nM

2. Ligand panel

See Fig. 2 and Fig. 3

3. Inhibitor panel

See Fig. 4

4. Stealth™ RNAi Testing

In progress

5. Cell culture and maintenance

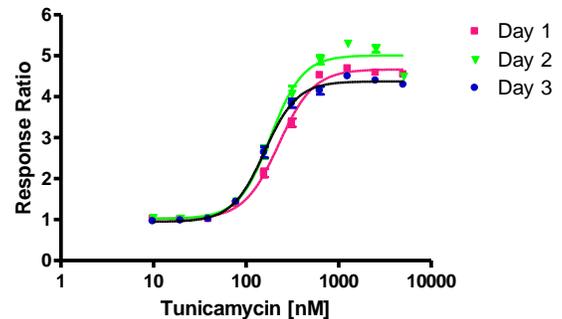
See Cell Culture and Maintenance Section and Table 1

Assay Testing Summary

6. Assay performance with variable cell number
7. Assay performance with variable DMSO concentration
8. Assay performance with variable substrate loading time
9. Assay performance with variable stimulation time

Primary Agonist Dose Response

Figure 1 — Tunicamycin dose response under optimized conditions

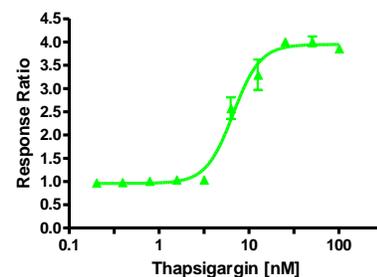


	Day 1	Day 2	Day 3
BOTTOM	1.016	1.023	0.9453
TOP	4.668	5.009	4.375
LOGEC50	2.355	2.265	2.201
HILLSLOPE	2.310	2.499	2.466
EC50	226.2	184.0	158.8

ESRE-*bla* HeLa cells were assayed on three separate days in 384-well assay format in Assay Medium at 5,000 cells/well. Following overnight incubation, serial dilutions of tunicamycin (EMD Biosciences, 654380) were applied to the wells (0.1 % final DMSO) for 5 h prior to loading the wells with LiveBLazer™-FRET B/G Substrate (1µM final concentration of CCF4-AM) for 2.5 hours. Emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader. Response Ratios were calculated by dividing the 460/530 ratios of the tunicamycin treated wells from the 460/530 ratios obtained with the untreated control wells (n = 16 for each data point).

Alternative Agonists

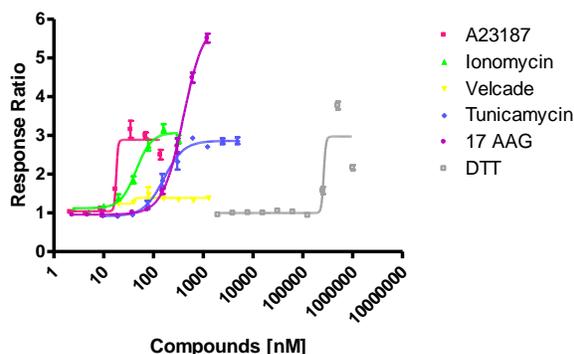
Figure 2 — Thapsigargin dose response under the optimized condition



	Thapsigargin
BOTTOM	0.9592
TOP	3.947
LOGEC50	0.8237
HILLSLOPE	2.886
EC50	6.663

ESRE-*bla* HeLa cells were assayed in 384-well assay format in Assay Medium at 5,000 cells/well. Following overnight incubation, serial dilutions of Thapsigargin (Sigma, T9033) were applied to the wells (0.1 % final DMSO) for 5 h prior to loading the wells with LiveBLazer™-FRET B/G Substrate (1µM final concentration of CCF4-AM) for 2.5 hours. Emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader. Response Ratios were calculated by dividing the 460/530 ratios of the thapsigargin treated wells from the 460/530 ratios obtained with the untreated control wells (n = 8 for each data point).

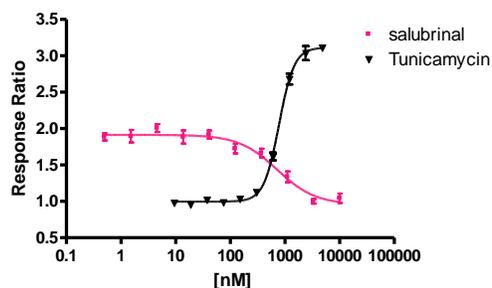
Figure 3 – Known ER stress inducing agent panel



	A23187	Ionomycin	Velcade	Tunicamycin	17 AAG	DTT
BOTTOM	1.040	1.120	1.236	0.9154	0.9586	0.9973
TOP	2.886	3.072	1.390	2.861	5.969	2.971
LOGEC50	1.251	1.651	1.602	2.209	2.599	5.417
HILLSLOPE	23.14	2.802	27.90	2.178	2.094	20.38
EC50	17.81	44.81	40.02	162.0	397.6	260929

ESRE-*bla* HeLa cells were assayed in 384-well assay format in Assay Medium at 5,000 cells/well. Following overnight incubation, serial dilutions of indicated agents were applied to the wells (0.1 % final DMSO) for 5 h prior to loading the wells with LiveBLazer™-FRET B/G Substrate (1µM final concentration of CCF4-AM) for 2.5 hours. Emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader. Response Ratios were calculated by dividing the 460/530 ratios of the treated wells from the 460/530 ratios obtained with the untreated control wells (n = 8 for each data point).

Figure 4 – Inhibitor Testing



	salubrinal	Tunicamycin
BOTTOM	0.9553	0.9962
TOP	1.915	3.113
LOGEC50	2.859	2.892
HILLSLOPE	-1.236	3.122
EC50	722.1	779.4

ESRE-*bla* HeLa cells were assayed in 384-well assay format in Assay Medium at 5,000 cells/well. Following overnight incubation, serial dilutions of salubrinal were applied to the wells (0.1 % final DMSO) for 30 minutes prior to the treatment with tunicamycin for 5 hours and loading the wells with LiveBLazer™-FRET B/G Substrate (1µM final concentration of CCF4-AM) for 2.5 hours. Emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader. Response Ratios were calculated by dividing the 460/530 ratios of the treated wells from the 460/530 ratios obtained with the untreated control wells (n = 8 for each data point).

Cell Culture and Maintenance

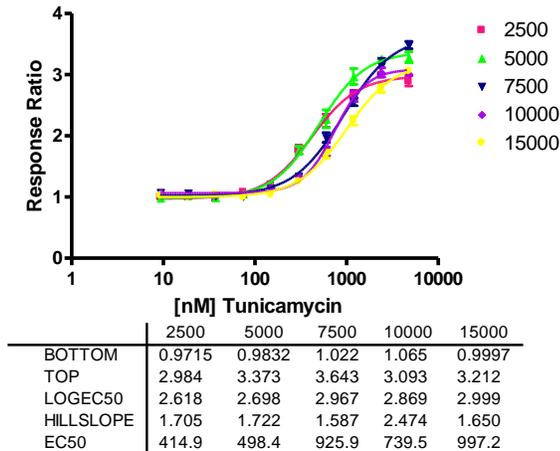
Thaw cells in Growth Medium without selection (Blasticidin) and culture them in Growth Medium with selection. Pass or feed cells 2-3 times a week and maintain them in a 37°C/5% CO₂ incubator. Maintain cells between 10% and 90% confluence. *Note:* We recommend passing cells for three passages after thawing before using them in the beta-lactamase assay. For more detailed cell growth and maintenance directions, please refer to protocol.

Table 1 – Cell Culture and Maintenance

Component	Growth Medium (-)	Growth Medium (+)	Assay Medium	Freeze Medium
DMEM with GlutaMAX™	500 mL	500 mL	—	—
OPTI-MEM	—	—	500 mL	—
Dialyzed FBS (dFBS) Do not substitute!	50 mL	50 mL	0.5 mL	—
HEPES (1 M)	12.5 mL	12.5 mL	—	—
NEAA (100x)	5 mL	5 mL	5 mL	—
Pen/Strep (100x)	5 mL	5 mL	5 mL	—
Na Pyruvate (100x)	—	—	5 mL	—
Blasticidin	—	5 µg/mL	—	—
Recovery™ Cell Culture Freezing Medium	—	—	—	100%

Assay Performance with Variable Cell Number

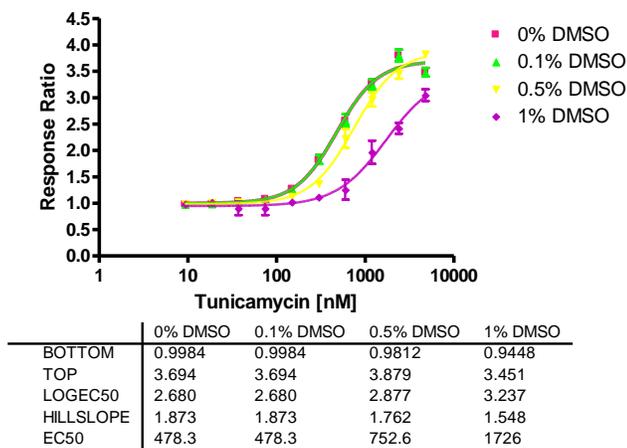
Figure 5 – Tunicamycin dose response with varying cell plating density



ESRE-*bla* HeLa cells were assayed in 384-well assay format in Assay Medium at indicated number of cells/well. Following overnight incubation, serial dilutions of tunicamycin were applied to the wells (0.1 % final DMSO) for 5 h prior to loading the wells with LiveBLAzer™-FRET B/G Substrate (1 μ M final concentration of CCF4-AM) for 2.5 hours. Emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader. Response Ratios were calculated by dividing the 460/530 ratios of the treated wells from the 460/530 ratios obtained with the untreated control wells (n = 8 for each data point).

Assay Performance with variable DMSO concentration

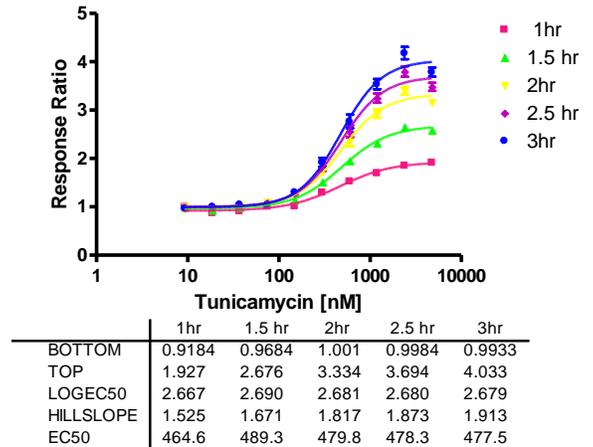
Figure 6 – Tunicamycin dose response with 0.1, 0.25, 0.5 and 1% DMSO.



ESRE-*bla* HeLa cells were assayed in 384-well assay format in Assay Medium at 5000 cells/well. Following overnight incubation, serial dilutions of tunicamycin were applied to the wells in the presence of indicated amount of final DMSO for 5 h prior to loading the wells with LiveBLAzer™-FRET B/G Substrate (1 μ M final concentration of CCF4-AM) for 2.5 hours. Emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader. Response Ratios were calculated by dividing the 460/530 ratios of the treated wells from the 460/530 ratios obtained with the untreated control wells (n = 8 for each data point).

Assay performance with Variable Substrate Loading Time

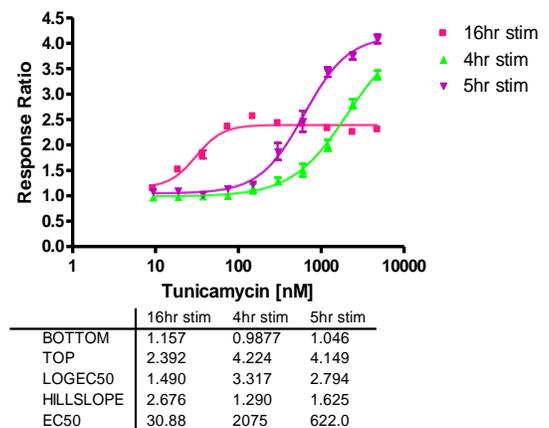
Figure 7 – Tunicamycin dose response with increasing substrate loading times



ESRE-*bla* HeLa cells were assayed in 384-well assay format in Assay Medium at 5000 cells/well. Following overnight incubation, serial dilutions of tunicamycin were applied to the wells (0.1 % final DMSO) for 5 h prior to loading the wells with LiveBLAzer™-FRET B/G Substrate (1 μ M final concentration of CCF4-AM) for indicated amount of time. Emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader. Response Ratios were calculated by dividing the 460/530 ratios of the treated wells from the 460/530 ratios obtained with the untreated control wells (n = 8 for each data point).

Assay performance with Variable Stimulation Time

Figure 8 – Tunicamycin dose response with varying stimulation times



ESRE-*bla* HeLa cells were assayed in 384-well assay format in Assay Medium at 5000 cells/well. Following overnight incubation, serial dilutions of tunicamycin were applied to the wells (0.1 % final DMSO) for 4, 5 and 16 hrs prior to loading the wells with LiveBLAzer™-FRET B/G Substrate (1 μ M final concentration of CCF4-AM) for 2.5 hours. Emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader. Response Ratios were calculated by dividing the 460/530 ratios of the treated wells from the 460/530 ratios obtained with the untreated control wells (n = 8 for each data point).

