Validation & Assay Performance Summary

CellSensor® TrkB-NFAT-*bla* CHO-K1 Cell Line

Cat. no. K1491

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

The Trk (tropomyosin-related kinase) family of neurotrophic receptor tyrosine kinases is comprised of three members, TrkA, TrkB, and TrkC, which can be activated by one or more of the neurotrophins NGF, BDNF, NT3 and NT4. Neurotrophin binding to their respective receptors can activate multiple signaling pathways (e.g. MAPK, PI3K, PKC). In addition each neurotrophin can activate the tumor necrosis factor receptor superfamily member, p75NTR (NGFR) and its downstream pathways and effectors (e.g. NFkB, Jun).

BDNF (brain-derived neurotrophic factor) and its receptor TrkB (also known as NTRK2) play a fundamental role in regulating neural development, survival, and synaptic activity and plasticity. TrkB is also a potential anti-cancer target since altered signaling through TrkB promotes tumor formation, survival, and metastasis of various cancers (e.g. neuroblastomas, multiple myelomas, and pancreatic ductal adenocarcinomas). Moreover, growing evidence indicates that BDNF and its receptor influence food intake and body weight control.

Cell Line Description

CellSensor® TrkB-NFAT-*bla* CHO-K1 cells contain a beta-lactamase reporter gene under control of the NFAT Response Element that has been stably integrated into CHO-K1 cells along with TrkB. TrkB-NFAT-*bla* CHO-K1 cells express bete-lactamase upon stimulation with brain-derived neurotrophic factor (BDNF). This cell line is a clonal population isolated by flow cytometry and has been tested for robust assay performance by assessing a variety of assay parameters.

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 BDNF EC_{50}	= 320 pM
Average Z'-Factor (EC_{100})	= 0.85
Average Response Ratio	= 17.5
Recommended cell no.	= 12,000 cells/well
Recommended [DMSO]	= up to 1 %
Stimulation Time	= 5 hours
Max. [Stimulation]	= 15 nM BDNF

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

Assay Testing Summary

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

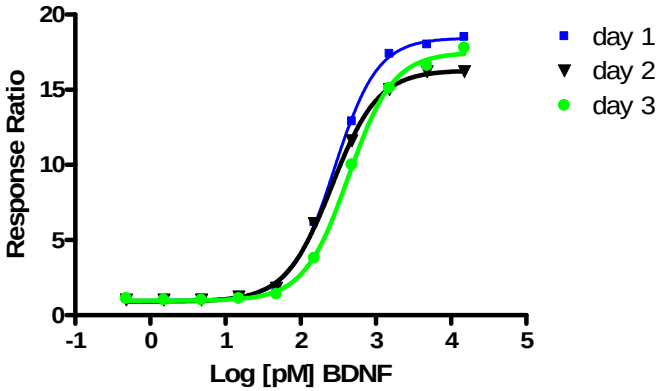
4. **Assay performance with variable DMSO concentration**

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

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

Primary Agonist Dose Response

Figure 1 — BDNF dose-response under optimized conditions



TrkB-NFAT-*bla* CHO-K1 cells were assayed on three separate days in 384-well assay format in growth medium at 12,000 cells/well. The cells were stimulated for 5 hours with a serial dilution of BDNF in the presence of 0.1 % DMSO prior to loading the wells with LiveBLAzer™-FRET B/G Substrate (1µM final concentration of CCF4-AM) plus Solution D for 2 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 obtained with each BDNF treatment by the 460/530 ratios obtained with the nontreated controls (n = 16 for each data point).

Cell Culture and Maintenance

Thaw cells in Growth Medium without selection (Blasticidin or Zeocin) 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% CO_2 incubator. Maintain cells between 5% and 95% confluence.

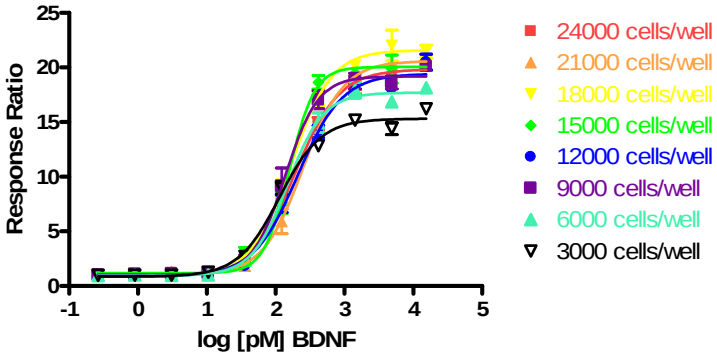
Note: We recommend passing cells for three passages after thawing before using them in the beta-lactamase assay. For more detailed cell growth and maintenance directions, please refer to protocol.

Table 1 – Cell Culture and Maintenance

Component	Growth Medium (–)	Growth Medium (+)	Assay Medium	Freeze Medium
DMEM with GlutaMAX™	500 mL	500 mL	500 mL	—
Dialyzed FBS (dFBS) **Do not substitute!**	50 mL	50 mL	50 mL	—
HEPES (1 M)	12.5 mL	12.5 mL	12.5 mL	
NEAA (100x)	5 mL	5 mL	5 mL	—
Pen/Strep (100x)	5 mL	5 mL	5 mL	—
Blasticidin	—	5 µg/mL	—	—
Zeocin	—	200 µg/mL	—	—
Recovery™ Cell Culture Freezing Medium	—	—	—	100%

Assay Performance with Variable Cell Number

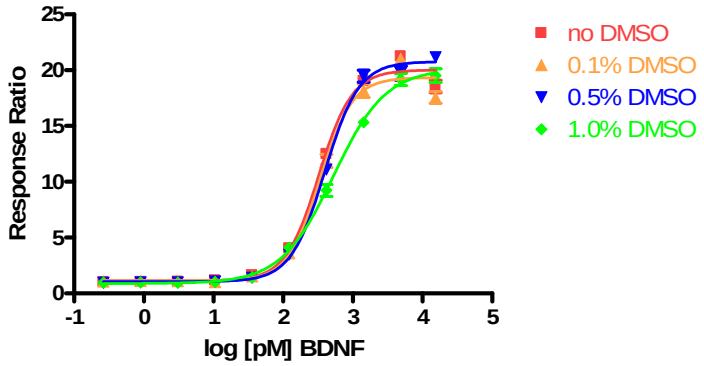
Figure 2— BDNF dose response with varying cell plating density



TrkB-NFAT-*bla* CHO-K1 cells were plated in 384-well assay format in growth medium at the indicated cell plating densities. The cells were stimulated for 4 hours with a serial dilution of BDNF in the presence of 0.1 % DMSO prior to loading the wells with LiveBLAzer™-FRET B/G Substrate (1µM final concentration of CCF4-AM) plus Solution D for 2 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 obtained with each BDNF treatment by the 460/530 ratios obtained with the nontreated controls (n = 4 for each data point).

Assay Performance with variable DMSO concentration

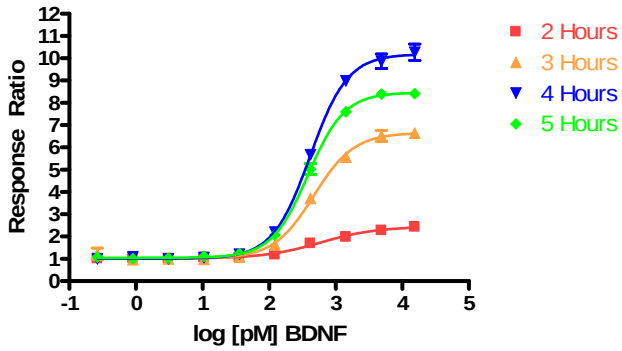
Figure 3 – BDNF dose response with 0, 0.1, 0.5 and 1% DMSO.



TrkB-NFAT-*bla* CHO-K1 cells were plated in 384-well assay format in growth medium at 12,000 cells/well The cells were stimulated for 4 hours with a serial dilution of BDNF in the presence of the indicated DMSO concentrations prior to loading the wells with LiveBLAzer™-FRET B/G Substrate (1µM final concentration of CCF4-AM) plus Solution D for 2 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 obtained with each BDNF treatment by the 460/530 ratios obtained with the nontreated controls (n = 8 for each data point).

Assay performance with Variable Stimulation Time

Figure 4 – BDNF dose response with varying stimulation times



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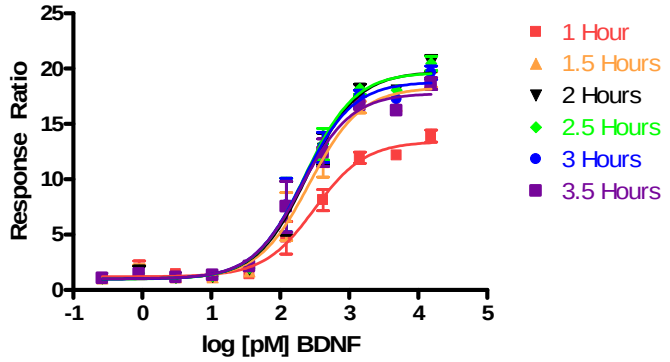
TrkB-NFAT-*bla* CHO-K1 cells were plated in 384-well assay format in growth medium at 12,000 cells/well. The cells were stimulated for the indicated times with a serial dilution of BDNF in the presence of 0.1 % DMSO prior to loading the wells with LiveBLAzer™-FRET B/G Substrate (1µM final concentration of CCF4-AM) plus Solution D for 2 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 obtained with each BDNF treatment by the 460/530 ratios obtained with the nontreated controls (n = 8 for each data point).

Assay performance with Variable Substrate Loading Time

Figure 5 – BDNF dose response with varying loading times



TrkB-NFAT-*bla* CHO-K1 cells were plated in 384-well assay format in growth medium at 12,000 cells/well. The cells were stimulated for 4 hours with a serial dilution of BDNF in the presence of 0.1 % DMSO prior to loading the wells with LiveBLAzer™-FRET B/G Substrate (1µM final concentration of CCF4-AM) plus Solution D for the indicated times. 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 obtained with each BDNF treatment by the 460/530 ratios obtained with the nontreated controls (n = 4 for each data point).