# Validation & Assay Performance Summary

## CellSensor®AP1-*bla* ME-180 Cell Line

Cat. no. K1661

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

Epidermal Growth Factor (EGF) is a peptide that induces cellular proliferation through the EGF receptor, which has a tyrosine kinase cytoplasmic domain, a single transmembrane domain and an extracellular domain involved in EGF binding and receptor dimerization. Inhibitors of the EGF receptor are being pursued as potential cancer therapies and EGF may stimulate wound healing. Mutation of the EGF receptor has been associated with cancer in humans. Proliferative effects of EGF signaling occur through several pathways, namely the activation of the ras and MAP kinase (MAPK) pathway. This in turn causes phosphorylation of transcription factors such as c-Fos to create AP-1 and ELK-1 that contribute to proliferation. Activation of STAT-1 and STAT-3 transcription factors by JAK kinases in response to EGF contributes to proliferative signaling. Phosphatidylinositol signaling and calcium release induced by EGF activate protein kinase C, another component of EGF signaling. Crosstalk of EGF signaling with other pathways indicates that the EGF receptor serves as a junction point between signaling systems.

## Cell Line Description

The CellSensor® AP-1-*bla* ME-180 cell line contains a beta-lactamase reporter gene under control of the Activator Protein-1 (AP-1) response element stably integrated into ME-180 cells. ME-180 cells are human cervical carcinoma cells. This cell line has also been tested for assay performance under variable conditions, including DMSO concentration, cell number, stimulation time and validated for Z' and $EC_{50}$ concentrations of Epidermal Growth Factor (EGF). Additional testing information using additional compounds, small molecule inhibitors and Stealth™ RNAi is also provided.

## Validation Summary

Testing and validation of this assay was evaluated using LiveBLAzer™-FRET B/G Substrate.

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

| | |
|---|---|
| EGF $EC_{50}$ | = 221 pg/ml |
| Z'-Factor ($EC_{100}$) | = 0.82 |
| Response Ratio | = 8.5 |
| Optimum cell no. | = 7.5K cells/well |
| Optimum [DMSO] | = up to 1% |
| Optimum Stim. Time | = 4-5 hours |
| Max. [Stimulation] | = 50 ng/mL |

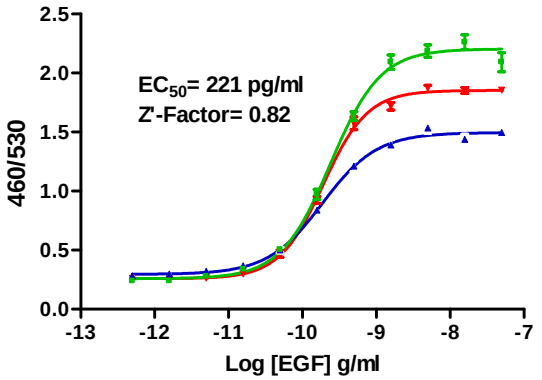
2. **Compound panel**
   *See Compound Panel Section*
3. **Small molecule inhibitor panel**
4. **Stealth™ RNAi testing**
5. **Cell culture and maintenance**
   *See Cell Culture and Maintenance Section and Table 1*

## Assay Testing Summary

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

## Primary Agonist Dose Response

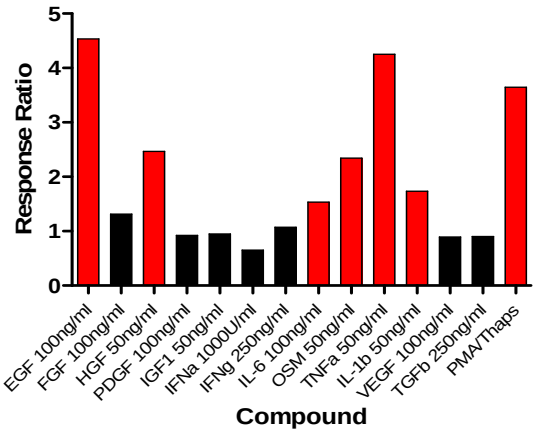
**Figure 1 — AP-1-*bla* ME-180 dose response to EGF under optimized conditions**




AP-1-*bla* ME-180 cells (7,500 cells/well) were assayed on three separate days, represented by the three curves shown on the graph. Cells were plated in a 384-well plate and stimulated with EGF (Sigma# 9644) 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 for the indicated concentrations of EGF (n=5 for each data point).

## Compound Panel

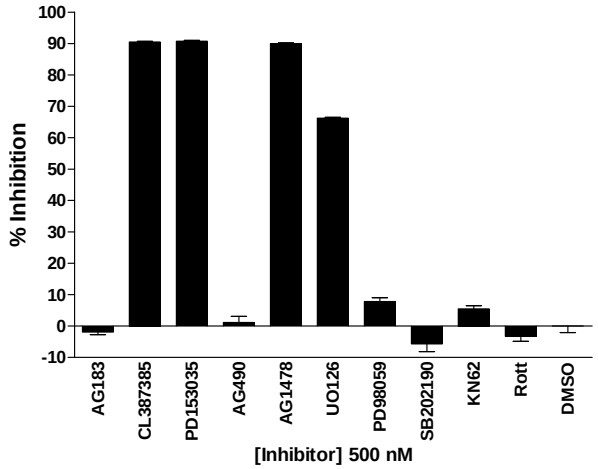
**Figure 2 — AP1-*bla* ME-180 response to various compounds**




AP-1-*bla* ME-180 cells treated with the listed compounds 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 for each compound.

## Small Molecule Inhibitor Response

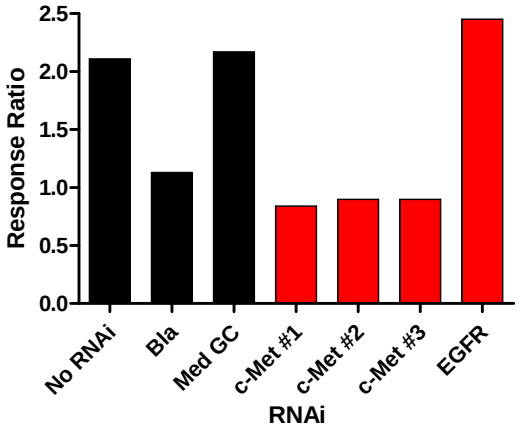
**Figure 3 — AP1-*bla* ME-180 response to various small molecule inhibitors**




AP-1-*bla* ME-180 cells treated with the listed compounds in the presence of 0.5% DMSO for 30 minutes. Cells were then treated with EGF (Sigma# 9644) for 5 hrs 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 converted to % inhibition relative to the negative control (DMSO).

## Stealth™ RNAi Testing

**Figure 4— AP1-*bla* ME-180 response to various Stealth™ RNAi and HGF stimulation**




AP-1-*bla* ME-180 cells were treated with the listed Stealth™ RNAi duplexes for 60 hrs. Cells were then stimulated with HGF for 5 hrs 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 Response Ratios plotted for each RNAi.

## Cell Culture and Maintenance

Thaw cells in Growth Medium without Blasticidin and culture them in Growth Medium with Blasticidin. Pass or feed cells at least twice a week and maintain them in a 37°C/5% $CO_2$ incubator. Maintain cells between 10% and 90% confluency. Do not allow cells to reach confluence.

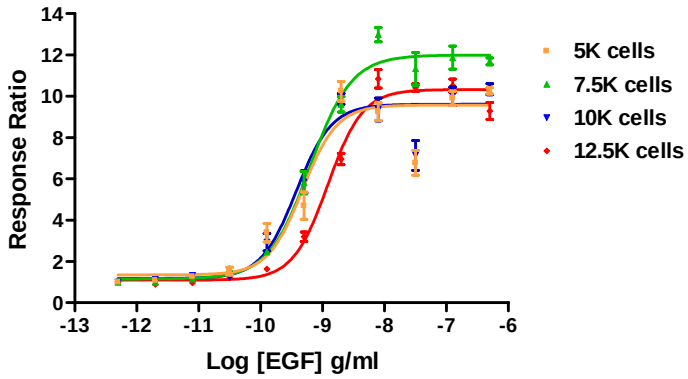
*Note:* We recommend passing cells for three passages after thawing before using them in the beta-lactamase assay. For optimal cell line performance, use dialyzed FBS (Invitrogen # 26400-010). For detailed growth and maintenance directions, refer to the protocol.

### Table 1 – Cell Culture and Maintenance

| Component | Growth Medium | Assay Medium | Freezing Medium |
|---|---|---|---|
| DMEM | 90% | — | — |
| Opti–MEM® | — | 99.5% | — |
| Dialyzed FBS **Do not Substitute!** | 10% | 0.5% | — |
| NEAA | 0.1 mM | 0.1 mM | — |
| Sodium pyruvate | 1 mM | 1 mM | — |
| HEPES (pH 7.3) | 25 mM | — | — |
| Penicillin (antibiotic) | 100 U/ml | 100 U/ml | — |
| Streptomycin (antibiotic) | 100 µg/ml | 100 µg/ml | — |
| Blasticidin (antibiotic) | 5 µg/ml | — | — |
| Recovery™ Cell Culture Freezing Medium | — | — | 100% |

## Assay Performance with Variable Cell Number

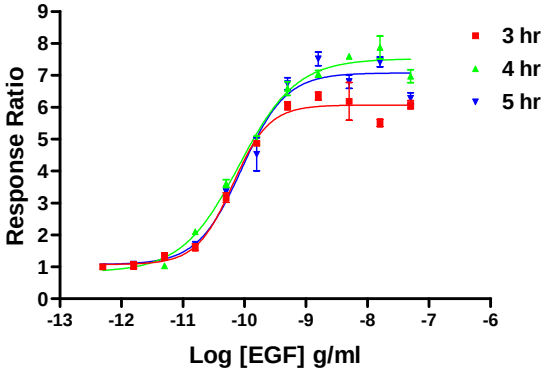
**Figure 5 — AP-1-*bla* ME-180 dose response to EGF using 5000, 7500, 10000, or 12,500K cells/well**




AP-1-*bla* ME-180 cells were plated at 5000, 7500, 10000, or 12,500 cells/well in a 384-well format. Cells were then stimulated with the indicated concentrations of EGF (Sigma# 9644) in the presence of 0.5% DMSO for 5 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate for 2.5 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 plotted for each cell number at the indicated concentrations of EGF(n=8 for each data point).

## Assay Performance with Variable Stimulation Time

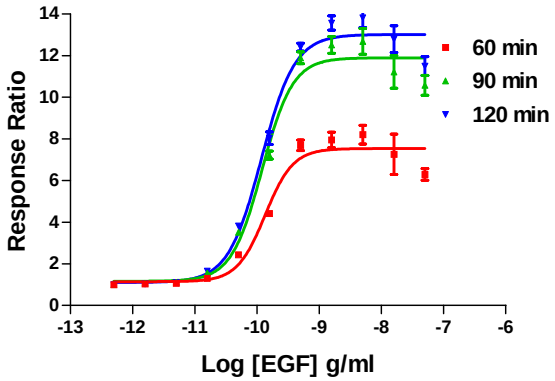
**Figure 6— AP-1-*bla* ME-180 dose response to EGF using 3, 4, and 5 hour stimulation times.**




AP-1-*bla* ME-180 cells (7,500 cells/well) were plated in a 384-well assay plate. Cells were then stimulated for either 3, 4, or 5 hrs with EGF (Sigma# 9644) in 0.5% DMSO and then loaded for 2.5 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 at the indicated concentrations of EGF (n=8 for each data point).

## Assay Performance with Variable Substrate Loading Time

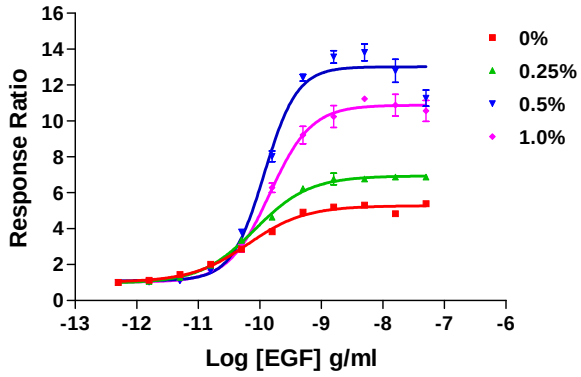
**Figure 7 — AP-1-*bla* ME-180 dose response to EGF with 1, 1.5, and 2 hour substrate loading times**




AP-1-*bla* ME-180 cells were plated at 7,500 cells/well in a 384-well format. Cells were then stimulated with the indicated concentrations of EGF (Sigma# 9644) 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, 2 or 2.5 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 at the indicated concentrations of EGF(n=8 for each data point).

## Assay Performance with Variable DMSO Concentration

**Figure 8 —AP-1-*bla* ME-180 dose response to EGF using 0, 0.25, 0.5 and 1% DMSO**




AP-1-*bla* ME-180 cells (7,500 cells/well) were plated in a 384-well plate and stimulated with the indicated concentrations of EGF (Sigma# 9644) using final DMSO concentrations ranging from 0% to 1%. Plates were stimulated for 5 hrs and loaded for 2.5 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 DMSO concentration at the indicated concentrations of EGF (n=8 for each data point).