

## InCELL Hunter™ U2OS BCL2-BAX Protein Binding Cell Line

**Catalog Number:** 96-0010C3 **Lot Number:** See Vial

**Contents:** 2 vials, 1 x 10<sup>6</sup> cells per vial in 1 mL

### Background

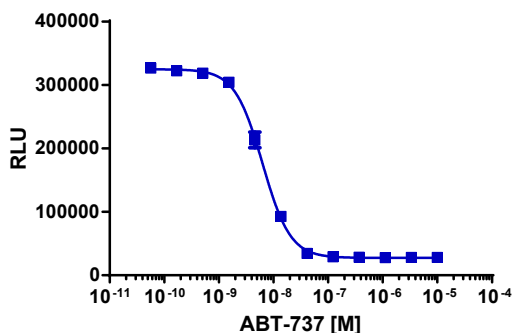
This InCELL Hunter assay measures intracellular compound-target engagement by detecting degradation of a partner protein that is ordinarily stabilized by its natural interaction with the target protein. The cell lines are engineered to co-express an untagged target protein, and an Enzyme Donor (ED) tagged partner protein. Either protein may be full length or truncated. Addition of inhibitors disrupting the interaction of the two proteins results in degradation of the ED-tagged protein. Addition of exogenous Enzyme Acceptor (EA), and buffer, lyses the cell and forces complementation of the ED and EA enzyme fragments. This results in the formation of a functional enzyme that hydrolyzes substrate to generate a chemiluminescent signal.

### Product Information

|  |   |  |                           |
|--|---|--|---------------------------|
| <b>Target Protein 1:</b>                   | BCL2  | <b>Target Protein 2:</b>                   | BAX                       |
| <b>Amino Acid Range:</b>                   | 1 - 239   | <b>Amino Acid Range:</b>                   | 52 - 72                   |
| <b>Accession #:</b>                        | NM_000633   | <b>Accession #:</b>                        | NM_138761                 |
| <b>Description:</b>                        | BCL2 B-cell CLL/lymphoma 2  | <b>Description:</b>                        | BCL2-associated X protein |
| <b>Target Tag 1:</b>                       | None  | <b>Target Tag 2:</b>                       | ePL                       |
| <b>Target Tag 1 located at (terminus):</b> | N/A   | <b>Target Tag 2 located at (terminus):</b> | N-Terminus                |
| <b>Target Species:</b>                     | Human   |  |                           |
| <b>Cell Type:</b>                          | U2OS  |  |                           |
| <b>Storage:</b>                            | Short term (<24 h): Store at -80°C; Long term (>24 h): Store in vapor phase of liquid nitrogen. |  |                           |

### Functional Performance

Cells were plated in a 384-well plate and incubated overnight at 37°C and 5% CO<sub>2</sub> to allow the cells to attach and grow. Cells were then stimulated with a control compound, using the assay conditions described below. Following treatment, signal was detected using the InCELL Hunter Detection Kit according to the recommended protocol using the conditions specified below. Please refer to page 2 for recommended assay reagents, detection reagents, and control compounds.



|   |         |
|---|---------|
| <b>Cell Number/Well:</b>                              | 10000   |
| <b>Cell Seeding Time (Hours):</b>                     | 24      |
| <b>Control Compound:</b>                              | ABT-737 |
| <b>Ligand Incubation Time (minutes):</b>              | 180     |
| <b>Ligand Incubation Temperature (°C):</b>            | 37      |
| <b>IC<sub>50</sub> of Inhibitor (nM):</b>             | 6.3     |
| <b>Signal:Background at compound E<sub>max</sub>:</b> | 11.7    |
| <b>1:4 EA Dilution Required?</b>                      | No      |
| <b>Detection Incubation Time (hours):</b>             | 1       |

### Passage Stability

This cell line has been confirmed to be stable through 10 passages with no significant drop in assay window or change in EC<sub>50</sub>.

### Mycoplasma Testing

This lot was tested and found to be free of mycoplasma contamination. Data available upon request.

### Required Materials

The following additional materials are required but not provided:

| Product Use*    | Product Description                    | Catalog Number |
|-----------------|--|----------------|
| Detection       | InCELL Detection Kit                   | 96-0079        |
| Cell Culture    | AssayComplete™ Cell Culture Kit-103    | 92-3103G       |
| Cell Plating    | AssayComplete™ Cell Plating 16 Reagent | 93-0563R16A    |
| Cell Detachment | AssayComplete™ Cell Detachment Reagent | 92-0009        |
| Cell Thawing    | AssayComplete™ Thawing Reagent T3      | 92-4103TR      |
| Cell Freezing   | AssayComplete™ Freezing Reagent F3     | 92-5103FR      |

\*Please inquire about our cell line-specific AssayComplete Starter Packs to get you started with your cell culture needs.

### Required Antibiotics

| Antibiotic Name             | Concentration (µg/mL) | Catalog Number |
|-----------------------------|-----------------------|----------------|
| AssayComplete™ Puromycin    | 0.25                  | 92-0028        |
| AssayComplete™ Hygromycin B | Not Applicable        | Not Applicable |
| AssayComplete™ G418         | 500                   | 92-0030        |

### Additional Ligand Information

**Control Compound:** ABT-737

**Vendor:** DiscoverX® (Catalog No. 92-1151)

For order placement or technical support, please call 1.866.448.4864 (North America) or +44.121.260.6142 (Europe) or e-mail [info@discoverx.com](mailto:info@discoverx.com). For additional information, please visit [discoverx.com](http://discoverx.com).

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