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Data Sheet

Myc Signaling Pathway
Myc Reporter (Luc) – HCT116 Cell line
Catalog #: 60520

Product Description
The Myc signaling pathway plays an important role in cell proliferation, differentiation, transformation and apoptosis. The c-Myc protein is a transcription factor that heterodimerizes with Max to regulate transcription of genes involved in proliferation, transformation and angiogenesis. Myc mutations have been linked to the development of a number of human cancers, including Burkitt's lymphoma, cervical, ovarian, breast, lung and pancreatic carcinoma, making Myc a promising therapeutic target.

The Myc Reporter – HCT116 cell line contains the firefly luciferase gene under the control of Myc responsive elements stably integrated into HCT116 cells, a human colon cancer cell line. HCT116 contains a mutated β-catenin which leads to the accumulation of β-catenin and constitutive activation of downstream Myc that induces the expression of Myc luciferase reporter. The cell line is validated for the inhibition of the expression of Myc luciferase reporter.

Applications
- Monitor Myc pathway activity.
- Screen for activators or inhibitors of the Myc pathway.

Format
Each vial contains 1.5 X 10^6 cells in 1 ml of 10% DMSO.

Storage
Immediately upon receipt, store in liquid nitrogen.

General culture conditions

Thaw Medium 7 (BPS Cat. #60185): McCoy's 5A medium (Hyclone #SH30200.01) with 10% FBS (Life technologies #26140-079), 1% Penicillin/Streptomycin (Hyclone SV30010.01).

Complete Growth Medium: Thaw Medium 7 (BPS Cat. #60185) plus 400 µg/ml of Geneticin (Life Technologies #11811031).

Cells should be grown at 37°C with 5% CO_2 using complete growth medium.

To thaw the cells, it is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, transfer to a tube containing 10 ml of pre-warmed Thaw Medium 7 **(no Geneticin)** and spin down the cells. After spin, re-suspend the cells in pre-warmed Thaw

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Medium 7 **(no Geneticin)**. Transfer the re-suspended cells to a T25 flask and incubate overnight in a 5% CO_2 incubator at 37°C. The next day, replace the growth medium with fresh Thaw Medium 7 Thaw Medium 7 **(no Geneticin)**, and continue growing culture in the CO_2 incubator at 37°C until the cells are ready to be split. At first passage, switch to complete growth medium **(contains Geneticin)**. Cells should be split before they reach complete confluence.

To passage the cells, rinse the cells with phosphate buffered saline (PBS) and detach cells from culture vessel with 0.05% Trypsin/EDTA. Add complete growth medium to quench the Trypsin reaction, transfer the cells to a tube, and spin down the cells. Re-suspend cells in complete growth medium and seed appropriate aliquots of cell suspension into new culture vessels. Sub-cultivation ratio: 1:15 to 1:30 weekly or twice a week.

To freeze down the cells, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with Trypsin/EDTA. Add complete growth medium and transfer to a tube, spin down cells, and resuspend in freezing medium (10% DMSO + 90% FBS). Place at -80°C overnight and place in liquid nitrogen the next day. Alternatively, vials may be placed directly in liquid nitrogen.

Functional Validation and Assay Performance
The following assays are designed for 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volume should be scaled appropriately.

Materials Required but Not Supplied

- ICG-001 (Selleckchem #S2662): an inhibitor of the Wnt/β-catenin pathway. Myc can be activated by the Wnt pathway.
- Assay medium: Opti-MEM I (Life Technologies #31985-062) + 0.5% FBS + 1% Non-essential amino acids + 1% sodium pyruvate + 1% penicillin/streptomycin
- 96-well tissue culture plate or 96-well tissue culture-treated, white clear-bottom assay plate
- ONE-Step™ Luciferase Assay System (BPS Cat. #60690). Other luciferase assay systems are also suitable.
- Luminometer

Mycoplasma testing
The cell line has been screened using the PCR-based VenorGeM® Mycoplasma Detection kit (Sigma-Aldrich) to confirm the absence of *Mycoplasma* species.

Inhibition of Myc reporter activity by inhibitor ICG-001 in Myc Reporter – HCT116 cells

Note: Set up each assay condition in at least triplicate.

1. Harvest Myc reporter (Luc)-HCT116 cells and seed cells at a density of 25,000 cells per well into white clear-bottom 96-well microplate in 100 µl of growth medium without Geneticin. Incubate the plate at 37°C with 5% CO_2 overnight.

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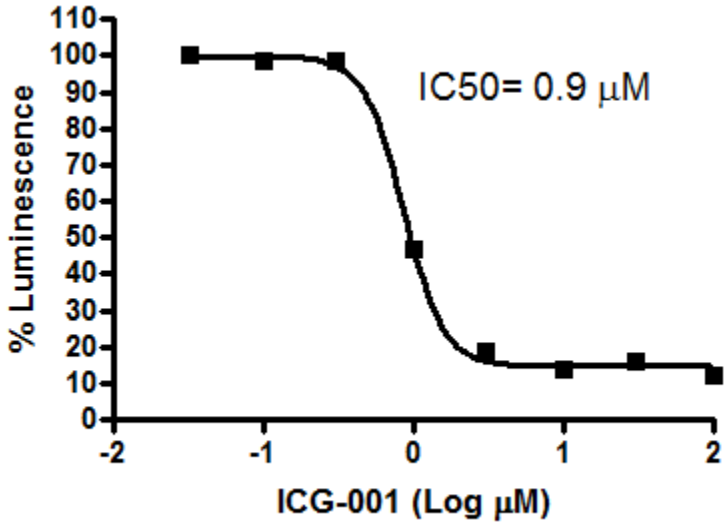
2. The next day, remove medium from the wells, and add 50 µl of assay medium containing threefold serial dilutions of ICG-001 to the sample wells. The final concentration of DMSO in the assay medium can be up to 0.1%.
   Add 50 µl of assay medium with DMSO to the control wells.
   Add 50 µl of assay medium with DMSO to cell-free control wells to determine background luminescence.

3. Incubate cells at 37°C with 5% CO_2 for 18 hours (overnight).

4. Perform luciferase assay using the ONE-Step™ Luciferase Assay System (BPS Cat. #60690): Add 50 µl of ONE-Step™ Luciferase reagent per well and rock at room temperature for ~10 to 15 minutes. Measure luminescence using a luminometer.
   If using other luciferase reagents from other vendors, follow the manufacturer's assay protocol.

5. Data Analysis: Obtain the background-subtracted luminescence by subtracting the average background luminescence (cell-free control wells) from the luminescence reading of all wells.

Figure 1. Dose response inhibition of constitutively active Myc reporter activity to inhibitor ICG-001 in Myc reporter (Luc)-HCT116 cells. The results are shown as percentage of luminescence. The background-subtracted luminescence of cells in the absence of ICG-001 was set at 100%.



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Reference:

Pelengaris S, *et al*. (2002) c-MYC: more than just a matter of life and death. *Nat. Rev. Cancer*. **2(10):** 764-76.

Related Products

Product name	Cat. #	Size
c-Myc	40453	100 µg
Myc Reporter Kit	60519	500 rxns
NF-κB Reporter Kit	60514	500 rxns
TCF/LEF Reporter Kit	60500	500 rxns
NK-κB Reporter (Luc) – HEK293 Cell Line	60650	2 vials
TCF/LEF Reporter (Luc) – HEK293 Cell Line	60650	2 vials
ISRE Reporter (Luc) – HEK293 Cell Line	60610	2 vials
AP1 Reporter (Luc) – HEK293 Cell Line	60405	2 vials
SRE Reporter (Luc) – HEK293 Cell Line	60406	2 vials
Gli Reporter (Luc) – NIH3T3 Cell Line	60409	2 vials
ONE-Step™ Luciferase Assay System	60690-1	10 ml
ONE-Step™ Luciferase Assay System	60690-2	100 ml

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