Description

The Myc Luciferase Reporter HCT116 Cell Line is a HCT116 cell line expressing firefly luciferase under the control of Myc responsive elements. These cells contain a mutated APC gene, which leads to the aberrant accumulation of β-catenin and constitutive expression of Wnt-responsive genes by the transcription factors TCF/LEF (T cell factor/lymphoid enhancer factor family) and CBP (Creb-binding protein).

The transcription factor Myc is an early response gene that is over-expressed as a result of β-catenin signaling in HCT116 cells, leading to constitutive expression of the Myc-dependent luciferase reporter. The cell line is validated for inhibition of Myc activation by ICG-001, an inhibitor of β-catenin/CBP-mediated gene transcription.

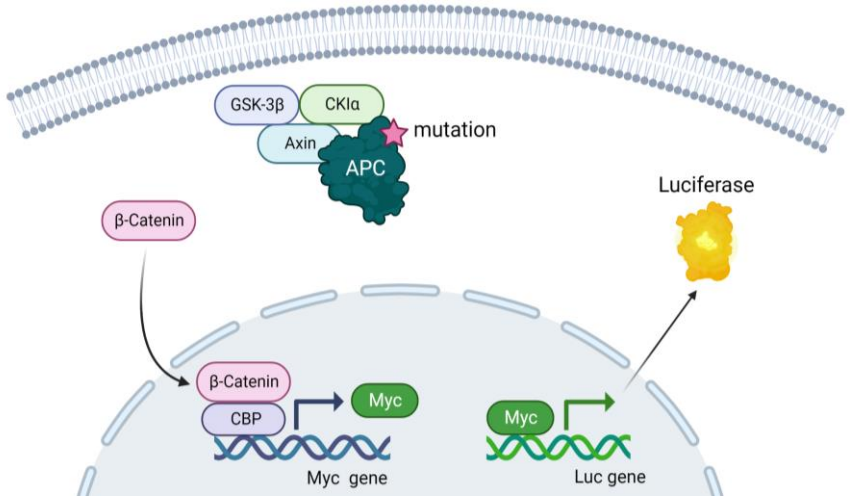

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Figure 1: Illustration of constitutive luciferase expression as a result of APC mutation and aberrant β-catenin signaling in Myc Luciferase Reporter HCT116 cells.

Background

Myc is a family of three transcription factors that controls cell proliferation, differentiation, transformation, and apoptosis. Translocation of the gene encoding c-Myc contributes to the development of Burkitt's lymphoma, whereas up-regulation of Myc genes has been observed in various human cancers including cervical, ovarian, breast, lung, and colon carcinoma. The aberrant expression of c-Myc in colon cancer is often due to an oncogenic mutation in the APC gene, which results in constitutive β-catenin/CBP-mediated gene transcription of the gene encoding c-Myc.

Application

- Monitor Myc transcriptional activity.
- Screen or titrate Myc regulators.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains >1 x 10^6 cells in 1 ml of Cell Freezing Medium (BPS Bioscience #79796)

Parental Cell Line

HCT116, Human Colorectal Carcinoma, epithelial-like cells, adherent

Mycoplasma Testing
The cell line has been screened to confirm the absence of Mycoplasma species.

Materials Required but Not Supplied

These materials are not supplied with the cell line but are necessary for cell culture and cellular assays. BPS Bioscience's reagents are validated and optimized for use with this cell line and are highly recommended for best results. Media components are provided in the Media Formulations section below.

Media Required for Cell Culture

Name	Ordering Information
Thaw Medium 7	BPS Bioscience #60185
Growth Medium 7B	BPS Bioscience #79545

Materials Used in Cellular Assay

Name	Ordering Information
Assay Medium 7B	BPS Bioscience #79718
ICG-001	Selleckchem #S2662
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
96-well white clear-bottom cell culture plate	
Luminometer	

Storage Conditions

Cells are shipped in dry ice and should immediately be thawed or stored in liquid nitrogen upon receipt. Do not use a -80°C freezer for long term storage. Contact technical support at support@bpsbioscience.com if the cells are not frozen in dry ice upon arrival.

Media Formulations
For best results, the use of validated and optimized media from BPS Bioscience is *highly recommended*. Other preparations or formulations of media may result in suboptimal performance.

Note: Thaw Media do *not* contain selective antibiotics. However, Growth Media *do* contain selective antibiotics, which are used to maintain selective pressure on the cell population expressing the gene of interest.
Cells should be grown at 37°C with 5% CO_2. BPS Bioscience's cell lines are stable for at least 10 passages when grown under proper conditions.

Media Required for Cell Culture

Thaw Medium 7 (BPS Bioscience #60185):
McCoy's 5A medium supplemented with 10% FBS, and 1% Penicillin/Streptomycin.

Growth Medium 7B (BPS Bioscience #79545):
McCoy's 5A medium supplemented with 10% FBS, 1% Penicillin/Streptomycin and 400 µg/ml of Geneticin.

Media Required for Functional Cellular Assay

Assay Medium 7B (BPS Bioscience #79718):
Opti-MEM I, supplemented with 0.5% FBS, 1% Non-essential amino acids, 1 mM sodium pyruvate, and 1% penicillin/streptomycin.

Cell Culture Protocol
Note: HCT116 cells are derived from human material. The use of adequate safety precautions is recommended.

Cell Thawing

1. Retrieve a cell vial from liquid nitrogen storage. Keep on dry ice until ready to thaw.

2. When ready to thaw, swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. Once cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire content of the vial to an empty 50 ml conical tube.
   Note: Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.

3. Using a 10 ml serological pipette, slowly add 10 ml of pre-warmed Thaw Medium 7 to the conical tube containing the cells. Thaw Medium 7 should be added dropwise while gently rocking the conical tube to permit gentle mixing and avoid osmotic shock.

4. Immediately spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 5 ml of pre-warmed Thaw Medium 7.

5. Transfer the resuspended cells to a T25 flask or T75 flask and incubate at 37°C in a 5% CO_2 incubator.

6. After 24 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw Medium 7 and continue growing in a 5% CO_2 incubator at 37°C until the cells are ready to passage.

7. Cells should be passaged before they are fully confluent. At first passage and subsequent passages, use Growth Medium 7B.

Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS) without Ca^{2+}/Mg^{2+}, and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 7B and transfer to a tube.

3. Spin down cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in Growth Medium 7B. Seed into new culture vessels at the desired sub-cultivation ratio of 1:5 twice/week.

Cell Freezing

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS) without Ca^{2+}/Mg^{2+}, and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 7B and count the cells.

3. Spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 4°C Cell Freezing Medium (BPS Bioscience #79796) at ~2 x 10^6 cells/ml.

4. Dispense 1 ml of cell aliquots into cryogenic vials. Place the vials in an insulated container for slow cooling and store at -80°C overnight.

5. Transfer the vials to liquid nitrogen the next day for storage.

Note: It is recommended to expand the cells and freeze at least 10 vials at an early passage for future use.

A. Validation Data

- 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.
- Perform the experiment in triplicates.
- The assay should include "Background Control", "Untreated Control" and "Test Inhibitor" conditions.

Assay Medium 7B (BPS Bioscience #79718):
Opti-MEM I, supplemented with 0.5% FBS, 1% Non-essential amino acids, 1 mM sodium pyruvate, and 1% penicillin/streptomycin.

Inhibition of Myc-dependent reporter activity by ICG-001

1. Seed Myc Luciferase Reporter HCT116 cells in **Thaw Medium 7** at a density of 25,000 cells/100 µl/well into a clear-bottom white 96-well plate. Keep wells without cells as "Background Control" (to determine background luminescence).
2. Incubate the plate at 37°C with 5% CO2 overnight.
3. The next day, prepare a threefold serial dilution of inhibitor ICG-001 (100 µl/well) in **Assay Medium 7B.** *Note: The final concentration of DMSO in the assay medium should not be over 0.1%.*
4. Remove the medium from the plate.
5. Add 100 µl of the inhibitor serial dilution to the "Test Inhibitor" wells.
6. Add 100 µl of Assay Medium 7B with 0.1% DMSO to the "Untreated Control" wells.
7. Add 100 µl of Assay Medium 7B with 0.1% DMSO to the "Background Control" wells.
8. Incubate the cells at 37°C with 5% CO_2 for approximately 18 hours (overnight).
9. Add 100 µl of ONE-Step™ Luciferase reagent per well.
10. Rock at room temperature for ~10 to 15 minutes.
11. Measure luminescence using a luminometer.

12. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells. Calculate the percent of luciferase activity:

$$\%\ Luciferase\ activity = \frac{Lumin.\,treated\ cells - ave.\,background}{Lumin.\,untreated\ cells - ave.\,background}$$

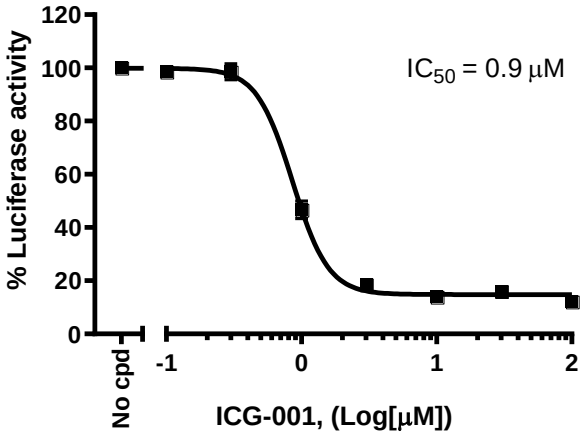

Figure 1: Inhibition of Myc-dependent reporter activity in the Myc luciferase Reporter HCT116 Cell Line by ICG-001.
Cells were treated with increasing concentrations of ICG-001 overnight. Luciferase activity was measured using the ONE-Step™ Luciferase Assay System (BPS Bioscience #60690). Results are shown as percentage of luciferase activity (compared to untreated control cells, set at 100%).

References

Pelengaris S, *et al*. 2002, *Nat. Rev. Cancer*. 2(10): 764-76.
Rennoll S, Yochum G., 2015 *World J Biol Chem.* 6(4): 290-300.

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Troubleshooting Guide

Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

Related Products

Products	*Catalog #*	*Size*
c-Myc, His-Tag Recombinant	40453	50 µg
Anti-Myc-Tag, Monoclonal	25012	100 µg
Myc Reporter Kit	60519	500 reactions

Version 110223