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Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

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See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

Description

The p53 Luciferase Reporter HCT116 Cell Line is an HCT116 cell line (expresses wild-type p53) that expresses Firefly luciferase under the control of the p53 response element. This cell line monitors the activity of the p53 signaling pathway. This cell line has been validated in activation assays in response to Mitomycin C, Nutlin-3, and Doxorubicin.

Background

p53 is a transcription factor and tumor suppressor very frequently mutated in human cancer and often termed "guardian of the genome". Activated by DNA damage, oxidative stress, or deregulated oncogene expression, p53 binds to a specific site in the promoter region of target genes and leads to the transcriptional activation of downstream genes involved in DNA repair, cell cycle arrest, senescence, and apoptosis. p53 levels are normally low, as the protein is targeted for degradation by MDM2 (mouse double minute 2). Upon activation by oxidative stress, or other cellular stress stimuli, p53 levels increase in the cells and the protein is activated. Inactivation of p53 promotes genome instability and directly contributes to cell transformation, and mutations in p53 contribute to 50% of the tumors. Using p53 as a therapeutic target requires regulation of p53 function to endogenous levels, since high levels of p53 seem to result in premature aging. p53 itself is regulated by multiple molecular mechanisms. An understanding of the pathways that regulate p53 is critical for the development of therapeutic modalities that restore its function and levels to endogenous levels.

Application

- Screen for inhibitors or activators of p53 signaling pathway in a cellular model.
- Monitor activation of the p53 signaling pathway.

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 cell line, 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 7C	BPS Bioscience #78076

Materials Used in Cellular Assay

Name	Ordering Information
Assay Medium 7A	BPS Bioscience #78673
Mitomycin C	BPS Bioscience #27763
Nutlin-3	BPS Bioscience #27711
Doxorubicin	Sigma #D1515
Clear-bottom, white 96-well tissue culture-treated plate	Corning #3610
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
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℃ 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 7C (BPS Bioscience #78076):
McCoy's 5A medium supplemented with 10% FBS and 1% Penicillin/Streptomycin plus 1 µg/ml of Puromycin.

Media Required for Functional Cellular Assay

Assay Medium 7A (BPS Bioscience #78673):
McCoy's 5A medium supplemented with 0.5% charcoal stripped FBS.

Cell Culture Protocol

Cell Thawing

1. Swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. As soon as the cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire contents of the vial to a tube containing 10 ml of pre-warmed Thaw Medium 7.
   Note: Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.

2. 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.

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

4. 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.

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

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 7C 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 7C.

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1/5 to 1/10 weekly or twice per week.

   Note: Just after thawing and when cells are at low density, the cells may grow at a slower rate. It is recommended to split the cells at a 1/4 ratio in those cases. After several passages, the cell growth rate increases, and the cells can be split using a higher ratio.

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 7C 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 suspension into each cryogenic vial. 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 long term storage.

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

Validation Data

- The following assay was designed for a 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volume should be scaled appropriately.
- This experiment measures the effect of compounds on reporter activation.
- All conditions should be performed in triplicate.
- The assay should include "Stimulated Cells" and "Background Control" and "Unstimulated" controls.

1. Seed p53 Luciferase Reporter HCT116 cells at a density of ~30,000 cells/well in 100 µl of Thaw Medium 7 into a clear-bottom, white 96-well plate. Leave empty wells as cell-free control wells ("Background Control").

2. Incubate the plate at 37°C in a 5% CO_2 incubator.

3. After 24 hours, carefully remove the medium from all wells.

4. Prepare the compounds of interest at the desired testing concentrations in Assay Medium 7A.

5. Add 100 µl of Assay Medium 7A containing the compounds of interest to the "Stimulated Cells" wells.

6. Add 100 µl of Assay Medium 7A to the "Unstimulated Cells" wells (to determine the luminescence from p53 Luciferase reporter HCT116 cells).

7. Add 100 µl of Assay Medium 7A to cell-free control wells ("Background Control").

8. Incubate cells at 37°C in a 5% CO_2 incubator overnight (~18 hours).

9. Add 100 µl of ONE-Step™ Luciferase reagent to each well and rock at Room Temperature (RT) for ~15 to 30 minutes.

10. Measure luminescence using a luminometer.

11. Data Analysis: Subtract the average background luminescence from the luminescence reading of all other wells. The fold induction of p53 luciferase reporter expression is the average background-subtracted luminescence of stimulated wells divided by the average background-subtracted luminescence of unstimulated control wells.

$$Fold\ induction = \frac{Luminescence\ of\ \ Stimulated\ Wells - avg.background}{Avg.Luminescence\ of\ Unstimulated\ Wells - avg.background}$$

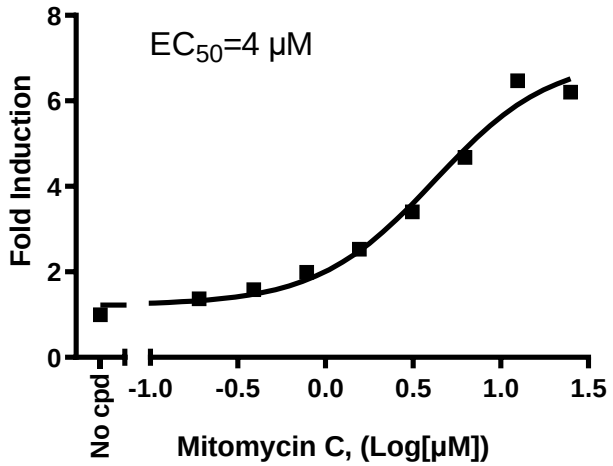

Figure 1: Reporter activation by Mitomycin C in the p53 Luciferase Reporter HCT116 Cell Line. p53 Luciferase Reporter HCT116 cells were incubated with increasing concentrations of Mitomycin C for 18 hours. Luciferase activity was measured using the ONE-Step™ Luciferase Assay System. The results are shown as fold induction of p53 luciferase reporter expression in relation to the activity of cells without treatment (unstimulated control).

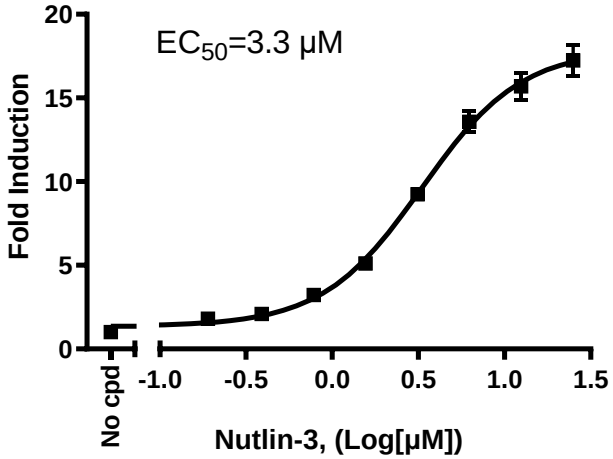

Figure 2: Reporter activation by Nutlin-3 in the p53 Luciferase Reporter HCT116 Cell Line. p53 Luciferase Reporter HCT116 cells were incubated with increasing concentrations of Nutlin-3 for 18 hours. Luciferase activity was measured using the ONE-Step™ Luciferase Assay System. The results are shown as fold induction of p53 luciferase reporter expression in relation to the activity of cells without treatment (unstimulated control).

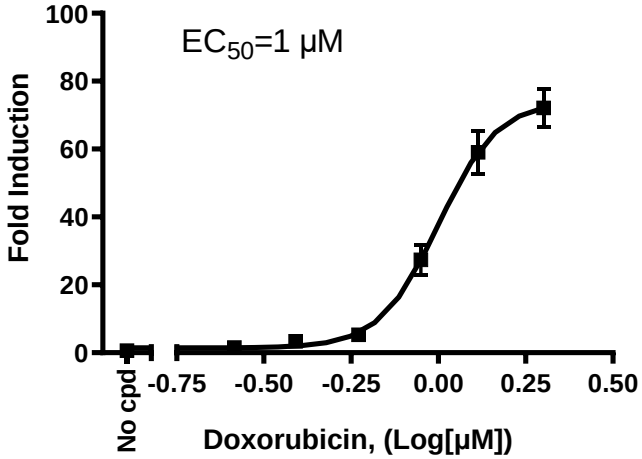

Figure 3: Reporter activation by Doxorubicin in the p53 Luciferase Reporter HCT116 Cell Line. p53 Luciferase Reporter HCT116 cells were incubated with increasing concentrations of Doxorubicin for 18 hours. Luciferase activity was measured using the ONE-Step™ Luciferase Assay System. The results are shown as fold induction of p53 luciferase reporter expression in relation to the activity of cells without treatment (unstimulated control).

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

References

Hafner A., *et al.*, 2019 *Nature Reviews Molecular Cell Biology* 20:199-210.

License Disclosure

Visit bpsbioscience.com/license for the label license and other key information about this product.

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*
p53 Luciferase Reporter Lentivirus	78666	500 µl x2
p53, GST-Tag Recombinant	40511	20 µg
p53 (Y220C), GST-Tag Recombinant	101610	20 µg
MDM2, GST-Tag Recombinant	100409	20 µg
MDM2 Intrachain TR-FRET Assay Kit	78302	384 reactions