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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

Firefly Luciferase HepG2 Cell Line is a liver cancer HepG2 cell line that expresses the firefly luciferase reporter driven by an EF1a promoter. This cell line has been generated by transduction with Firefly Luciferase Lentivirus (EF1A Promoter/ Hygromycin) (BPS Bioscience #78740-H).

This cell line has been validated in co-culture assays.

Background

HepG2 cell line is a human hepatoma cell line derived from a hepatocellular carcinoma. HepG2 cells have an epithelial-like morphology and a high proliferation rate. This cell line is commonly used in drug metabolism, hepatotoxicity, and liver disease research due to its ability to perform many liver-specific functions *in vitro.* Studies have shown that glypican-3 (GPC3) is significantly up-regulated in HepG2 cell line, making it an excellent cell line for studying the role of GPC3 in hepatocellular carcinoma and potentially develop targeted therapies. Firefly luciferase has been used as a sensitive reporter to study a wide range of biological responses. It was first cloned from the North American *Photinus pyralis* and catalyzes the oxidation of D-luciferin, in the presence of ATP and magnesium, emitting yellow light. This reaction has a high quantum yield, and both luciferase and luciferin have low toxicity. These characteristics contributed to Firefly luciferase becoming a commonly used tool. The use of firefly luciferase as reporter allows for easy readouts.

Application

- Use as target cells in CAR-T or CAR-NK co-culture killing assays.
- *In vitro* and *in vivo* bioluminescence imaging.

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

HepG2, human hepatoma 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 1	BPS Bioscience #60187
Growth Medium 1F	BPS Bioscience #79540

BPS Bioscience
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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 1 (BPS Bioscience #60187):

MEM supplemented with 10% FBS, 1% non-essential amino acids, 1mM Sodium pyruvate, 1% Penicillin/Streptomycin.

Growth Medium 1F (BPS Bioscience #79540):

MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin plus 100 µg/ml Hygromycin B.

Cell Culture Protocol

Note: Note: HepG2 cells are derived from human material and thus the use of adequate safety precautions is recommended.

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 1**.**

   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 1.

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

4. After 48-72 hours of culture, check for cell viability, change to fresh Thaw Medium 1, 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 reach 100% confluency. Switch to Growth Medium 1F for passage.

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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 0.25% Trypsin/EDTA following volumes recommended for the cell vessel being used.

2. Once the cells have detached, add Growth Medium 1F 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 1F.

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

Cell Freezing

1. Aspirate the medium, wash the cells with PBS without Ca^{2+}/Mg^{2+}, and detach the cells from the culture vessel with 0.25% Trypsin/EDTA following volumes recommended for the cell vessel being used.

2. Once the cells have detached, add Growth Medium 1F and transfer to a tube.

3. Spin down cells at 300 *x g* for 5 minutes.

4. Remove the medium and resuspend the cell pellet in 4°C Cell Freezing Medium (BPS Bioscience #79796) at a density of ~2 x 10^6 cells/ml.

5. 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.

6. 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

Cytotoxicity assay of Anti-GPC3 CAR-T Cells using Firefly Luciferase HepG2 Cell Line as the target cells

This cell line was validated in a co-culture cytotoxicity assay. For details about the exact protocol used to evaluate the cytotoxicity function of Anti-GPC3 CAR-T Cells (#82494) refer to Anti-GPC3 CAR-T Cells datasheet.

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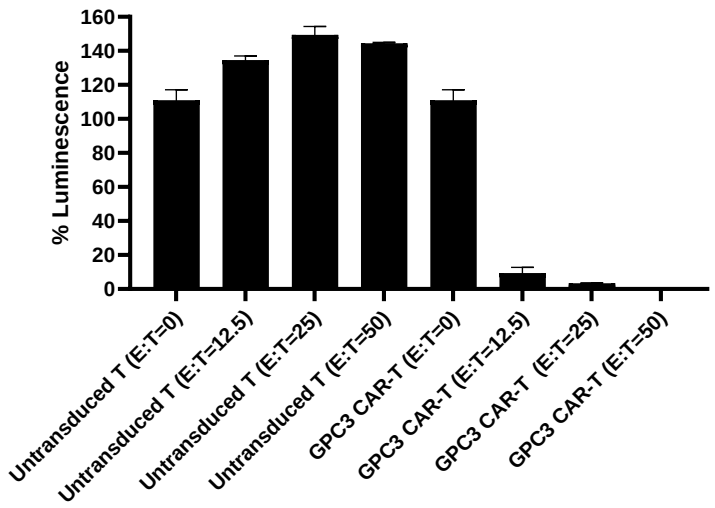

Figure 1. Luciferase-based cytotoxicity assay of Anti-GPC3 CAR-T Cells using Firefly Luciferase HepG2 Cell Line as target cells.
Anti-GPC3 CAR-T effector cells (#82494) were thawed and co-cultured with Firefly Luciferase HepG2 cells (#82490) as the target cells for 24 hours at the indicated E:T ratios. The assay was performed in parallel with untransduced T cells as a negative control. The lysis of the target cells after 24 hours was determined by measuring luciferase activity with ONE-Step™ Assay System (#60690). The average background luminescence was subtracted from the luminescence reading of all wells. Results are expressed as Percent of Luminescence, with "Firefly Luciferase HepG2 cells" alone set to 100%. The luminescence signal is proportional to the amount of living cells remaining in the co-culture population.

Data is representative.

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Troubleshooting Guide
Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please email visit https://bpsbioscience.com/contact.

Related Products

Products	*Catalog #*	*Size*
Firefly Luciferase Lentivirus (G418, Hygromycin and Puromycin)	79692	500 µl x 2
Firefly Luciferase Lentivirus EF1A Promoter/Geneticin, Hygromycin, or Puromycin)	78740	500 µl x 2
Firefly Luciferase-eGFP Lentivirus (G418) or (Puromycin)	79980	500 µl x 2
Firefly Luciferase-eGFP Lentivirus (EF1A Promoter/Geneticin, Hygromycin, or Puromycin)	78741	500 µl x 2

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