HepG2-GFP-luc

CSI411Hu12

Instruction manual

FOR RESEARCH USE ONLY

NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1st Edition (Revised in Apr, 2024)

[DESCRIPTION]

HepG2 is a cell line exhibiting epithelial-like morphology that was isolated from a hepatocellular carcinoma of a 15-year-old, White, male youth with liver cancer. HepG2-GFP-luc cells carry GFP and luciferase genes through lentiviral transfection, and can stably express firefly luciferase and green fluorescent protein. The cell line was stable and could be cultured for 30 generations without adding antibiotics. It can be used as a positive control in the detection of firefly luciferase activity, and can also be used in live animal imaging experiments.

Synonyms: HepG2-GFP-LUC; HepG2/GFP/LUC; HepG2/GFP/luc

I. Introduction

Host Cell: HepG2

Plasmid vector: pLVX-Luc2-P2A-AcGFP1-puro

Target Gene / Reporter(s): Green Fluorescent Protein; Firefly Luciferase

Resistance gene: Puromycin

Stability: 30 passages(in-house test, that not means the cell line will be instable beyond the passages we tested.)

Ⅱ. Description of Host Cell Line

Organism: Homo sapiens, human

Tissue: Liver

Disease: Carcinoma; Hepatocellular

Age: 15 years

Gender: Male

Morphology: epithelial-like

Growth properties: Adherent

[PROPERTIES]

Cell activity: >85% (Viability by Trypan Blue Exclusion).

Formulation: Frozen 1 mL or T25 flask.

Biosafety: Negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast and fungi.

Applications: For research use only. It is not approved for human or animal use, or for application in clinical diagnostic procedures.

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Size: >5×10^5cell/vial

[STORAGE]

Upon receiving, check all containers for leakage or breakage. directly and immediately transfer the cells from dry ice to liquid nitrogen and keep the cells in liquid nitrogen until they are needed for experiments.
Form & Buffer: Supplied as solution form in frozen stock solution, containing 90%FBS+10%DMSO.
Storage conditions: liquid nitrogen

[USAGE]

Culture conditions:

Complete growth medium：DMEM+10%FBS+1%Penicillin-Streptomycin Solution+10μg/ml Puromycin
Temperature: 37℃
Condition: 95% air, 5% carbon dioxide

Resistance screening:

1. The cell was stably transfected with GFP+luc, and its fluorescence intensity gradually decreased with the increase of cell passage times. If the fluorescence intensity needs to be maintained, puromycin can be added for re-screening. The screening concentration of purinomycin was 2μg/ml.
2. It is recommended to pass the cells for at least 3 generations after receiving them, and then screen them after freezing and retaining the species.
3. For initial cell screening, it is recommended to add complete medium with a final concentration of 1μg/ml puromycin for maintenance culture of 2-3 days. The concentration of puromycin was gradually increased to a maximum drug concentration of 10μg/ml, and the culture was maintained.

Cell recovery:

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the cap out of the water. The thawing time is about 2 minutes.
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by spraying with 75% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.
3. Transfer the vial contents to a centrifuge tube containing 9.0mL complete culture medium. and spin at approximately 1000 rpm for 5 minutes.
4. Resuspend cell pellet with the recommended complete medium . and dispense into a T25 culture flask.
5. Incubate the culture at 37°C, 5% CO_2 in a suitable incubator.

Cell passage:

1. Cell passage when cell growth at 85-95%.
2. Remove and discard culture medium and wash with PBS 1-2 times.
3. Add 1.0 mL of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 2 to 3 minutes. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal). Stop digestion by adding 2-3 ml of complete medium containing 10% serum. Make it a single cell suspension.

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4. Add the fresh medium to resuspend the cells. Unless otherwise stated, the recommended ratio of primary cells is 1/3-1/4.

[Shipping]

Dry ice.

[IMPORTANTNOTE]

1. This product is intended for laboratory research use only. It is not intended for any animal or human therapeutic use, any human or animal consumption, or any diagnostic use.
2. To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C. Storage at -70°C will result in loss of viability.
3. After cell recovery, please take regular microscopic examination and photos to record the growth status of cells.
4. Read the instructions carefully, and keep and operate in strict accordance with the instructions. If you observe abnormalities or have questions about cell culture operations, please contact us in time.

[Figure]

1. HepG2-GFP-luc cells screened by puromycin could still stably express GFP at a high level after 30 generations of continuous culture in complete medium without puromycin, and there was no significant difference in cell morphology between HepG2-GFP-luc cells and untransfected cells.

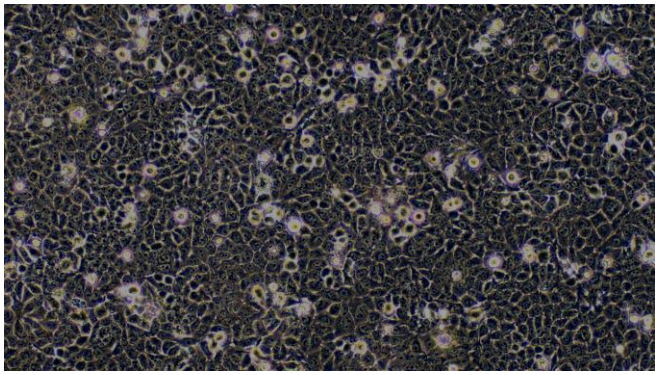

Figure 1

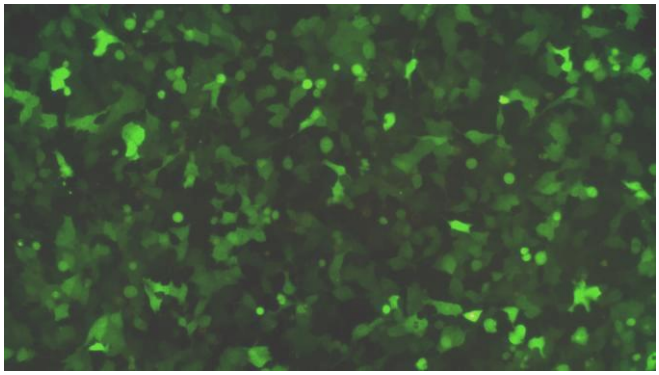

Figure 2

Figure 1 Morphology of HepG2-GFP-luc (Optical microscope,100x)

Figure 2 Morphology of HepG2-GFP-luc (Fluorescence microscope,100x)

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2. Assay of luciferase activity in vitro

HepG2 and HepG2-GFP-luc were taken, supernatant medium was removed, and 2ml of D-Luciferin working solution was added into the cells respectively. The luminescence was detected and image analysis was performed. The results showed that HepG2-GFP-luc cells could stably and highly express luciferase.

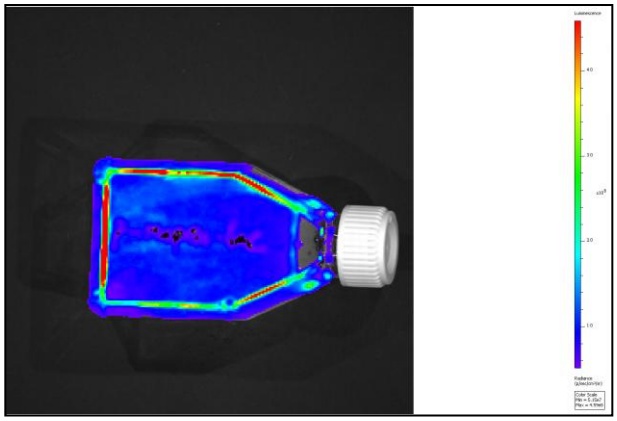

Figure 3

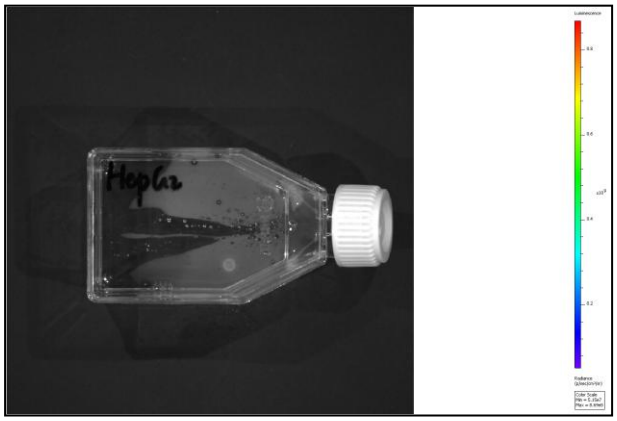

Figure 4

Figure 3 Bioluminescence in vitro of HepG2-GFP-luc

Figure 4 Bioluminescence in vitro of HepG2

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