HepG2 Cell Line Producing Hepatitis B Virus, Genotype A2

Catalog No. NR-56528

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For research use only. Not for use in humans.

Contributor:

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Manufacturer:

BEI Resources

Product Description:

HepG2 cells producing hepatitis B virus (HBV), genotype A2 (HepG2-GtA2) is a stable cell line designed by transfecting plasmids with an insert of replicon-competent 1.3x length HBV, genotype A2 genome and a hygromycin marker into HepG2 cells capable of HBV replication. HepG2-GtA2 can be passaged *in vitro* and *in vivo* for functional and biological studies.[1]

Material Provided:

Each vial contains approximately 1 mL of cell culture suspension frozen in freeze medium [80% Dulbecco's MEM (DMEM); 10% fetal bovine serum (FBS) and 10% dimethylsulfoxide (DMSO) cryopreservative]. Sufficient cells are provided to initiate at least one new culture. The cell count, expressed as cells per vial, is shown on individual Certificates of Analysis for each lot.

Packaging/Storage:

NR-56528 was packaged aseptically in screw-capped plastic cryovials. The product should be stored at -100°C or colder, preferably in the vapor phase of a liquid nitrogen freezer. Storage at -70°C will result in loss of viability.

Note: Do not under any circumstances store vials at temperatures warmer than -100°C. Storage under these conditions will result in the death of the culture.

To ensure the highest level of viability, the vial should be thawed, and the culture initiated as soon as possible upon receipt. Any warming of the product during shipping and transfer must be avoided, as this will adversely affect the viability of the product after thawing. For transfer between freezers and shipping, the cells may be placed on dry ice for brief periods, although use of a portable liquid nitrogen carrier is preferred. Please read the following recommendations prior to reconstituting this material.

Safety Precautions:

When handling frozen vials, it is highly recommended that protective gloves, lab coat and full-face mask be worn. Even brief exposure to the ultra-cold temperature can cause tissue damage from frostbite. Also, some vials may slowly fill with liquid nitrogen if they have been immersed during cryogenic storage. When thawing, the liquid nitrogen may rapidly expand as it changes to gas, breaking the vial or cap with explosive force, sending debris flying with enough velocity to cause injury. Store and use in areas with adequate ventilation.

Thawing and Growth:

Note: Extended incubation and sub-culturing may be required to produce sufficient cells for downstream applications.

Prior to thawing the cells, prepare cell growth media (CGM), with and without hygromycin B (refer to Appendix I and II). The CGMs are formulated for use in an aerobic atmosphere with 5% CO_2.

Rapidly thaw the vial of cells in a 37°C water bath. To reduce the risk of contamination, keep the cap and O-ring of the vial out of the water and repeatedly check the cap for tightness during thawing. Remove from the water bath immediately when thawed. Dry the vial with a sterile wipe, decontaminate using a wiper soaked with 70% isopropyl alcohol and let the vial air dry. Aseptically open the vial, remove the vial contents and add to a centrifuge tube containing 4 mL of CGM without antibiotic. Centrifuge the cell suspension at 125 × g for 8 to 10 minutes at 18°C to 25°C. Discard the supernatant and resuspend the cell pellet in 10 mL of pre-warmed CGM without antibiotic. Transfer the cell suspension into a 75 cm^2 tissue culture flask. Incubate the new culture at 37°C in an aerobic atmosphere with 5% CO_2 for 48 hours, then remove the CGM without antibiotic and replace with an equal amount of CGM with hygromycin B.

Sub-culture procedure:
After trypsinizing the monolayer using standard methods, aseptically transfer the contents of the flask to a centrifuge tube containing an equal volume of CGM without antibiotic. Centrifuge the cell suspension at 125 × g for 8 to 10 minutes at 18°C to 25°C. Discard the supernatant and resuspend the cell pellet in an equal amount as the original volume of pre-warmed CGM without antibiotic. Add cell suspension to as many 75 cm^2 tissue culture flasks as needed at a sub-cultivation ratio of 1:2 to 1:4 (a seeding density of 4×10^4 to 6×10^4 cells/cm^2 is recommended). Incubate the new culture at 37°C in an aerobic atmosphere with 5% CO_2 for 48 hours, then remove the CGM without antibiotic and replace with an equal amount of CGM with hygromycin B.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: HepG2 Cell Line Producing Hepatitis B Virus, Genotype A2, NR-56528."

Biosafety Level: 2

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories (BMBL). 6th ed. Washington, DC: U.S. Government Printing Office, 2020.

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References:

1. Zhang, M., et al. “Infection Course, Virological Features and IFN-α Responses of HBV Genotypes in Cell Culture and Animal Models.” J. Hepatol. 75 (2021): 1335-1345. PubMed: 34363922.

Support Provided by NIAID

Appendix I: Cell Growth Medium without Antibiotic for Cell Line Initialization (First 48 hours)

DMEM	90%
FBS	10%

Appendix II: Cell Growth Medium with Hygromycin B for Cell Line Maintenance

DMEM	90%
FBS	10%
Hygromycin B	500 µg/mL

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