



**HEP2B MHCC-97H | 305442**

**Viruses** HBV (HBV)

**Mutational profile** BRD7 p.Glu277Glyfs\*18 (c.830\_831delAG) KEAP1 p.Pro445Glnfs\*13 (c.1334delC) TP53 p.Glu51Ter (c.151G>T)

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**Culture Medium** DMEM 4.5 g/l Glucose 4 mM L-Glutamine 3.7 g/l NaHCO3 1.0 mM Sodium Pyruvate (100 mg/ml 820)

**Supplements** 10% FBS

**Dissociation Reagent** Trypsin

**Subculturing** Seed cells into fresh medium containing 10% FBS. When cells reach confluence, harvest cells by trypsinization.

**Seeding density** 1.5 x 10<sup>4</sup> cells/cm<sup>2</sup>

**Freeze medium** DMEM (10% FBS) + 10% DMSO

**Thawing and Culturing Cells**

1. Thaw cells rapidly in a 37°C water bath. Transfer cells to a pre-warmed medium.
2. Centrifuge cells at 300 x g for 3 minutes. Remove supernatant and wash cells with PBS.
3. Resuspend cells in fresh medium containing 10% FBS. Seed cells into a 25 cm<sup>2</sup> flask.
4. Allow cells to attach for 24 hours. Change medium to fresh medium containing 10% FBS.
5. Once cells are established, reduce FBS concentration to 5% for expansion.
6. For passaging, trypsinize cells and seed into a new flask at a density of 1.5 x 10<sup>4</sup> cells/cm<sup>2</sup>.
7. Monitor cell growth and confluency. Harvest cells when reaching 70-80% confluency.
8. For freezing, wash cells with PBS and resuspend in freeze medium. Seed into a cryovial.

