## *HepG2 culture conditions*

**Medium**: DMEM + 10% FBS + 1% pen-strep. Given the there are many formulations of DMEM and different versions of serum, we would like to provide the catalog numbers of the stuff that we are using: DMEM - HyClone cat. no. SH30022.02 & serum - HyClone cat. no. SH30070.03. Both could be purchased from VWR.

## **Procedure:**

1. Frozen cells should be thawed into a  $175 \text{ cm}^2$  flask containing 30 ml of medium and incubated @37C, 5% CO<sub>2</sub> and allowed to attach and fill out the dish. Change the media the next day.

2. Trypsinize with 0.05% or 0.25% trypsin. Split 1:8 – 1:16.

3. Change the media twice a week. Make sure it does not turn orange or yellow. For production, we grow these cells either in 15 cm dishes or (more commonly) cell-stacks. The yields are somewhat higher in dishes due to more complete trypsinization. The stacks are easier to handle on the other hand.

4. Grow to 75% confluence.

To see photographs of HepG2 cells, please go to

<u>http://www.atcc.org/common/images/Cells/HB-8065\_mg1.jpg</u>. Cells in the high density photograph were grown to approximately 90% confluence. The Resources Working Group will try to obtain and distribute a photograph of cells growing at 75% confluence.

## **Comments:**

These cells grow in foci which eventually merge together.