

## Handling Freshly Isolated Hepatocytes in Suspension

Important Note: Triangle Research Labs ships all hepatocytes, plated and in suspension, in a cold preservation medium at 4°C. This medium becomes cytotoxic at 6°C and is not suitable for hepatocyte culture. Before using your hepatocytes, please remove this medium and exchange it with plating or culture medium, following the directions in the protocol below.

### Protocol for Handling Fresh Hepatocytes in Suspension upon Receipt of Shipment

1. Remove tube of hepatocytes from packaging. Slowly invert tube several times to homogeneously suspend the cells.
2. Centrifuge hepatocytes at 4°C. For human hepatocytes, centrifuge at 100g for 8 min. For animal hepatocytes, centrifuge at 60g for 4 min.
3. Carefully aspirate the shipping medium off the pellet of cells (if preferred, you may leave ~1mL to prevent disrupting the pellet).
4. Resuspend the hepatocyte pellet in maintenance media (for suspension assays) or plating media (to plate hepatocytes). Add 1 mL for every million cells you expect to recover, adjusting for experimental conditions with higher or lower concentrations of cells.
5. Determine viability and yield of your hepatocytes by using the Trypan Blue exclusion method.
6. For suspension assays, adjust the cell density to meet your experimental design (generally  $0.5-2.0 \times 10^6$  live cells/mL).
7. For plated assays, use the tables below to adjust the live cell density to meet your experimental design. Note: For 96-well plates, add 50µL of blank plating media to each well followed by 50µL of cell stock. This uniformly disperses hepatocytes across the plating surface.

Table 1. Seeding Density (millions of cells/mL)

Plate Format	Human	Rat	Dog	Mouse	Cynomolgus
6-well	0.850	0.750	0.950	0.350	0.950
12-well	0.750	0.670	0.850	0.320	0.850
24-well	0.650	0.600	0.750	0.300	0.750
48-well	0.550	0.500	0.650	0.270	0.650
96-well	0.850	0.750	0.950	0.350	0.950

Table 2. Cell Volume Per Well (mL)

Plate Format	Cell Volume Per Well (mL/well) – All Species
6-well	2.0mL/well
12-well	1.0mL/well
24-well	0.50mL/well
48-well	0.20mL/well
96-well	0.050mL blank media/well + 0.050mL/well