

Assay Methods Hepatocyte Protocols

Determination of Hepatocyte Cell Concentration, Recovery and Viability by Trypan Blue Exclusion

The protocol below describes the procedure for determining the percentage cell viability, viable cell concentration per mL and total cell recovery for BD Gentest™ CryoHepatocytes once the cells are thawed and recovered.

Note: Follow standard sterility practices for cell culture.

Materials and Equipment:

- Biosafety hood
- Microscope
- Hemacytometer
- 2 mL Pipet (BD Cat. No. 357507)
- 0.4% Trypan Blue Solution
- 1X Phosphate buffer saline (PBS)
- Thawed and recovered BD Gentest CyroHepatocytes (Note: work quickly and do not leave hepatocytes at room temperature for extended periods of time.)

Procedure

1. Prepare a 1:10 dilution of Trypan Blue Solution with 1X PBS.
2. Make two 1:5 dilutions of the cell suspension using the diluted trypan blue solution (e.g., 40 μ L of diluted trypan blue solution + 10 μ L of cells into a 1.7 mL centrifuge tube).
3. Wait for approximately one minute, then immediately pipette 10 μ L from each tube into a separate chambers of a hemacytometer. Do not let cells sit in trypan blue before counting.
4. Observe the cells under the microscope (10X magnification objective lens).
5. Count the total number of live cells (clear cells) and the dead cells (blue cells) in quadrants; A, B, C, D, for each chamber (each quadrant is comprised of 16 smaller squares and is defined by triple lines, the middle of which is the boundary).
6. Calculate the % viability and average cells/ml (cells/mL * 10⁶) concentration below.

A		B
D		C

7. Calculate the % viability and average cells/ml (cells/mL * 10⁶) concentration below.

	Cell # Chamber I	Cell # Chamber II	% Viable (I)	% Viable (II)	c/mL*e6 (I)	c/mL*e6 (II)	Avg. Viable	Avg. Cells mL*e6
Dead								
Viable								

8. % Viability = {number live cells/ number total cells(live+dead)} x 100
9. Viable Cells/mL = (Viable Cell number in the 4 quadrants)/4 x 5 (the cell dilution factor) x 10⁴ (the volume of liquid per quadrant)
10. Total viable cell recovery = viable cells/mL x total volume of resuspended cells