Triangle Research Labs – Now Part of Lonza

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Cell Counting Protocol - Trypan Blue Exclusion Method

To determine cell viability and viable cell yield with the Trypan Blue Exclusion Method, follow the directions below.

1. Add 50µL of 0.4% Trypan Blue Solution to 350µL of media. Or use pre-aliquoted 1:8 Trypan Blue:stock solution.
2. Add 100µL of cell stock. This makes a final 1:5 cell:stock dilution. This will be referred to as the 'dilution factor' in the formula below.
3. Determine cell viability using the formula below.

Live cell count	/	Total cell count	=	Viability
	/		=	%

4. Determine total viable cell yield using the formula below.

Viable cell count	/	Quadrants counted	x	Dilution factor	x	Hemocytometer factor	x	Current volume (mL)	=	Viable cell yield
	/		x	5	x	10,000	x		mL =	

5. Use the formulas below to determine the volume of medium to add to your current cell stock to achieve the desired cell density (can be found on Table 2). The desired cell density varies for suspension assays but is most commonly 1.0×10^6 cells/mL. Use plating medium if plating your hepatocytes. Use maintenance medium for suspension assays.

Viable cell yield		Desired cell density*		Total volume needed
x 10^6 cells	/	x 10^6 cells/mL	=	mL

Total volume needed	-	Current volume	=	Volume to add to cell stock
mL -		mL=		mL

*Table 2. Desired Cell Density by Species and Plate Format

Species	6-well	12-well	24-well	48-well	96-well
	Cell Density (10^6cells/mL)				
Human, Rat, Dog	0.9 – 1.1	0.8 – 1.0	0.7 – 0.9	0.6 – 0.8	0.9 – 1.1
Monkey	1.1 – 1.3	1.0 – 1.2	0.9 – 1.1	0.8 – 1.0	1.1 – 1.3
Mouse	0.5 – 0.7	0.4 – 0.6	0.3 – 0.5	0.2 – 0.4	0.5 – 0.7