



ECLIPSE BIOINNOVATIONS

ECLIP: PREPARATION AND UV CROSSLINKING OF SUSPENSION CELLS

Required materials:

1. UV crosslinker with 254-nm wavelength UV bulbs (UVP CL-1000 Ultraviolet Crosslinker or equivalent)
2. Liquid nitrogen (sufficient to submerge tubes in appropriate container)
3. 1x DPBS (*Corning cat# 21-031-CV* or equivalent)
4. Trypan blue stain (*ThermoFisher Scientific, cat# 15250-061* or other equivalent live cell counting assay)
5. Standard cell counting system (hemocytometer or automated cell counter)

Cell viability validation (prior to crosslinking):

- a. Use Trypan blue stain (*ThermoFisher Scientific, cat# 15250-061* or other equivalent live cell counting assay to assay cell viability)
- b. Cell viability should be > 95% to ensure intact RNA

Preparation of suspension cells:

- a. Pool all cells per biosample (if multiple plates)
- b. Transfer cells with media to 50mL conical tube(s)
- c. Centrifuge at 200 x g for 5 minutes at room temperature
- d. Aspirate spent media

Wash cells:

- a. Resuspend the pellet(s) in 25mL of 1x DPBS at room temperature
- b. Count cell concentration (either with automated cell counter or hemocytometer)
- c. Spin down remaining sample in 50mL conical tube(s) at 200 x g for 5 minutes at room temperature
- d. Aspirate supernatant
- e. Resuspend cells to no more than 20×10^6 cells per mL

UV crosslinking:

- f. Aliquot at most 60×10^6 cells (re-suspended in 1x DPBS) in at least 3mL total volume to a standard 10cm tissue culture grade plate.
 - **Note:** Ensure the cells are evenly dispersed and the plate is fully covered (3mL should be sufficient volume).
- g. Place the tissue culture plate on leveled ice or a cooling block pre-chilled to 4°C
- h. Place the above (plate plus ice or cooling block) into the UV cross-linker.
 - **Notes: Ensure the plate is leveled**
 - **Remove tissue culture plate lid** for cross-linking
- i. Cross-link at 254-nm UV with an energy setting of 400 mJoules/cm²
 - **Note:** this is a setting of **4000** on many cross-linkers which display values in 0.1 mJoules/cm²
- j. After crosslinking is completed, transfer cells to a 50mL conical tube
- k. Wash plate once with 7mL of 1x DPBS and add to the same 50mL tube



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- l. Count cell concentration (either with automated cell counter or hemocytometer)
 - **Note:** ensure cells are re-suspended well before counting
- m. Centrifuge the 50mL conical tube at 200 x g for 5 minutes at room temperature.
- n. Aspirate and discard supernatant.
- o. Resuspend in the desired amount for flash freezing
 - Typically 20×10^6 cells per mL.
- p. Transfer desired amount into 1.5mL Eppendorf Safe-Lock Tubes (or equivalent)
 - Typically 1mL of 20×10^6 cells per mL.
- q. Spin down at 200 x g for 5 minutes at room temperature.
- r. Aspirate the supernatant and freeze by submerging the epi-tubes completely in liquid nitrogen.
- s. After frozen (at least 30 seconds), remove from the liquid nitrogen and store at -80°C .