



ECLIP: PREPARATION AND UV CROSSLINKING OF ADHERENT 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

Wash cells:

- a. Aspirate spent media
- b. Wash the plate gently with 1x DPBS at room temperature (15mL for a 15cm plate)
- c. Aspirate media
- d. Add enough 1x DPBS to just cover the plate (5mL for a 15cm plate)
* **Note:** If all plates are at equal cell density, one plate can be sacrificed for counting – this plate would be dissociated (with trypsin, accutase, or equivalent) and cell number (per plate) counted at this stage. **This is recommended for cell types that require chemical dissociation** enzymes to dissociate and be properly counted, as this is not recommended post-crosslinking

UV crosslinking:

- e. Place the tissue culture plate on leveled ice or a cooling block pre-chilled to 4°C
- f. 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
- g. 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
- h. While keeping the cells on ice, use a cell scraper (*Corning cat# CLS3010-10EA*) to scrape the plate
- i. Transfer the cells to a 50mL conical tube
- j. Wash plate once with 10mL of 1x DPBS and add to the same 50mL tube
- k. Gently resuspend until the sample is homogeneous



ECLIPSE BIOINNOVATIONS

- l. Count cell concentration (either with automated cell counter or hemocytometer)
 - **Note:** ensure cells are re-suspended well before counting
 - **Note:** for cells that do not easily dissociate into single cells, a separate plate of cells can be counted instead (see step d *note above)
- 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