Preparation and UV Crosslinking of Adherent Cells

Thank you for trusting Eclipsebio with supporting your RNA research and drug development goals. This document describes how to crosslink adherent cells in preparation for an eCLIP project.

If you have any questions, please contact us at services@eclipsebio.com.

Required Materials

- ☐ UV crosslinker with 254-nm wavelength UV bulbs (UVP CL-3000 Ultraviolet Crosslinker or equivalent)
- ☐ Standard cell counting system (hemocytometer or automated cell counter)
- ☐ Centrifuge
- ☐ Cell scraper (Corning cat# CLS3010-10EA or equivalent)
- ☐ 1x DPBS (Corning cat# 21-031-CV or equivalent)
- ☐ Trypan blue stain (Thermo Fisher Scientific cat# 15250061 or equivalent live cell counting assay)
- ☐ Chemical dissociation enzymes (trypsin, Accutase, or equivalent if necessary)
- ☐ Ice or cooling block
- ☐ Liquid nitrogen or dry ice (sufficient amount to submerge tubes, in an appropriate container)
- ☐ Standard 15 cm tissue culture grade plate
- ☐ Standard 50 mL conical tubes
- ☐ Standard 15 mL conical tubes
- ☐ Eppendorf LoBind 1.5 mL tubes (Fisher Scientific cat# 13-698-791 or equivalent)

1. Cell Viability Validation

Perform this step prior to crosslinking

- ☐ Use Trypan blue stain and cell counting system to count the number of viable cells.
- ☐ Cell viability should be >95% to ensure intact RNA.

2. Wash Cells

- ☐ Aspirate spent media.
- ☐ Wash the plate gently with chilled 1x DPBS (15 mL for a 15 cm plate).
- ☐ Aspirate media.
- ☐ Add enough chilled 1x DPBS to just cover the plate (5 mL for a 15 cm plate).

Note: if all plates are at equal cell density, one plate can be sacrificed for cell counting at this stage. **This is recommended for cell types that require chemical dissociation enzymes** (trypsin, Accutase, or equivalent) to dissociate and be properly counted, as this is not recommended post-crosslinking.

3. UV Crosslinking

- [ ] Place the tissue culture plate on leveled ice or a cooling block pre-chilled to 4ºC.
- [ ] Place the plate plus ice or cooling block into the UV crosslinker. **Ensure the plate is level and remove the tissue culture plate lid before crosslinking.**
- [ ] Crosslink at 254-nm UV with an energy setting of 400 mJoules/cm^2. **Check the specific values of your crosslinker, many display with units of 0.1 mJoules/cm2 and the setting would be 4000.**
- [ ] While keeping the cells on ice, use a cell scraper to scrape the plate.
- [ ] Transfer the cells to a 15 mL conical tube.
- [ ] Wash the plate once with 5 mL of chilled 1x DPBS and add to the same 15 mL conical tube
- [ ] Centrifuge the 15 mL conical tube at 250 x g for 5 minutes at 4ºC.
- [ ] Aspirate and discard the supernatant.
- [ ] Resuspend in the desired amount of chilled 1x DPBS (typically 20 x 10^6 cells per mL) for flash freezing.
- [ ] Transfer the desired amount (typically 1 mL of 20 x 10^6 cells per mL) into 1.5 mL Eppendorf LoBind Tubes (or equivalent).
- [ ] Spin down at 250 x g for 5 minutes at 4ºC.
- [ ] Aspirate the supernatant and freeze by submerging the tubes completely in liquid nitrogen or on dry ice.
- [ ] After frozen (at least 30 seconds), remove from the liquid nitrogen or dry ice and store at -80ºC.