Preparation and UV Crosslinking of Suspension Cells

Thank you for trusting Eclipsebio with supporting your RNA research and drug development goals. This document describes how to crosslink cells in suspension 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
- ☐ 1x DPBS (Corning cat# 21-031-CV or equivalent)
- ☐ Trypan blue stain (Thermo Fisher Scientific cat# 15250061 or equivalent live cell counting assay)
- ☐ 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. Preparation of Suspension Cells

- ☐ Pool all cells per biosample (if multiple plates).
- ☐ Transfer cells with media to 50 mL conical tube(s).
- ☐ Centrifuge at 250 x g for 5 minutes at 4ºC.

3. Wash Cells

- ☐ Aspirate spent media.
- ☐ Resuspend the pellet(s) in 25 mL of chilled 1x DPBS.
- ☐ Count cell concentration (either with an automated cell counter or hemocytometer).
- ☐ Spin down remaining sample in 50 mL conical tube(s) at 250 x g for 5 minutes at 4ºC.
- ☐ Aspirate supernatant.
- ☐ Resuspend cells to no more than 12 x 10^6 cells per mL.

4. UV Crosslinking

- [ ] Aliquot a minimum of 10 x 10^6 and a maximum of 60 x 10^6 resuspended cells in at least 5 mL total volume of 1x DPBS to a standard 15 cm tissue culture grade plate. **Ensure cells are evenly dispersed and the plate is fully covered.**
- [ ] 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.**
- [ ] After crosslinking is completed, 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.