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 or dry ice (sufficient to submerge tubes, in appropriate container)
3. 1x DPBS (*Corning cat# 21-031-CV or equivalent*)
4. Trypan blue stain (*Thermo Fisher 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 (*Thermo Fisher 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 chilled 1x DPBS (15mL for a 15cm plate)
c) Aspirate media
d) Add enough chilled 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:

a) Place the tissue culture plate on leveled ice or a cooling block pre-chilled to 4°C
b) Place the above (plate plus ice or cooling block) into the UV cross-linker

*** Note:** Ensure the plate is level. **Remove** tissue culture plate lid before cross-linking.

c) Cross-link at 254-nm UV with an energy setting of 400 mJoules/cm^2

***Note:** this is a setting of 4000 on many crosslinkers which display values in 0.1 mJoules/cm^2

d) While keeping the cells on ice, use a cell scraper (Corning cat# CLS3010-10EA) to scrape the plate
e) Transfer the cells to a 15mL conical tube
f) Wash plate once with 5mL of chilled 1x DPBS and add to the same 15mL tube
g) Centrifuge the 15mL conical tube at 250 x g for 5 minutes at 4°C
h) Aspirate and discard supernatant
i) Resuspend in the desired amount of chilled 1x DPBS for flash freezing
   - Typically 20×10^6 cells per mL
j) Transfer desired amount into 1.5mL Eppendorf Safe-Lock Tubes (or equivalent)
   - Typically 1mL of 20×10^6 cells per mL
k) Spin down at 250 x g for 5 minutes at 4°C
l) Aspirate the supernatant and freeze by submerging the LoBind tubes completely in liquid nitrogen or on dry ice
m) After frozen (at least 30 seconds), remove from the liquid nitrogen (or dry ice) and store at -80°C