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 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

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 250 x g for 5 minutes at 4°C

Wash cells:

a) Aspirate spent media
b) Resuspend the pellet(s) in 25mL of chilled 1x DPBS
c) Count cell concentration (either with automated cell counter or hemocytometer)
d) Spin down remaining sample in 50mL conical tube(s) at 250 x g for 5 minutes at 4°C
e) Aspirate supernatant
f) Resuspend cells to no more than 12 $x10^6$ cells per mL

UV crosslinking:

a) Aliquot a minimum of $10x10^6$ and a maximum of $60x10^6$ cells (re-suspend in 1x DPBS) in at least 5mL total volume to a standard 15cm tissue culture grade plate

*** Note:** Ensure the cells are evenly dispersed and the plate is fully covered (5mL should be a sufficient volume)

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

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

d) 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

e) After crosslinking is completed, 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