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UV Crosslinking of Suspension Cells for eCLIP

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ABSTRACT

Profiling of RNA binding protein targets in vivo provides critical insights into the mechanistic roles they play in regulating RNA processing. The enhanced crosslinking and immunoprecipitation (eCLIP) methodology provides a framework for robust, reproducible identification of transcriptome-wide protein-RNA interactions, with dramatically improved efficiency over previous methods. Here we provide a step-by-step description for UV crosslinking of suspension cells to prepare them for eCLIP experiments.

MATERIALS

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 (*Thermo Fisher Scientific, Cat# 15250-061 or other equivalent live cell counting assay*)
5. Standard cell counting system (hemocytometer or automated cell counter)

BEFORE START INSTRUCTIONS

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

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We use this protocol and it's working

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Prepare Suspension Cells

- 1 Pool all cells per biosample (if multiple plates)
- 2 Transfer cells with media to 50mL conical tube(s)
- 3 Centrifuge at 200g for 5 minutes at room temperature
- 4 Aspirate spent media

Wash Cells

- 5 Resuspend the pellet(s) in 25 mL of 1x DPBS at room temperature.
- 6 Count cell concentration (either with automated cell counter or hemocytometer)
- 7 Spin down remaining sample in 50ml conical tube(s) at 200g for 5 minutes at room temperature.
- 8 Aspirate supernatant
- 9 Resuspend cells to no more than 20×10^6 cells per mL

UV Crosslinking

- 10 Aliquot at most 60×10^6 cells (re-suspended in 1x DPBS) in at least 3 mL total volume to a standard 10cm tissue culture grade plate.
  - **Note:** Ensure the cells are evenly dispersed and the plate is fully covered (3 mL should be sufficient volume).
- 11 Place the tissue culture plate on leveled ice or a cooling block pre-chilled to 4°C
- 12 Place the above (plate plus ice or cooling block) into the UV cross-linker.
  - **Note:** Ensure the plate is leveled.

- Remove tissue culture plate lid for cross-linking.

- 13 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
- 14 After crosslinking is completed, transfer the cells to a 50 mL conical tube
- 15 Wash plate once with 7mL of 1x DPBS and add to the same 50 mL tube.
- 16 Gently resuspend until the sample is homogeneous
- 17 Count cell concentration (either with automated cell counter or hemocytometer)
  - **Note:** ensure cells are re-suspended well before counting
- 18 Centrifuge the 50 ml conical tube at 200g for five minutes at room temperature.
- 19 Aspirate and discard supernatant.
- 20 Resuspend in the desired amount for flash freezing
  - Typically 20×10⁶ cells per mL.

- 21 Transfer desired amount into 1.5 mL Eppendorf Safe-Lock Tubes (or equivalent)
  - Typically 1mL of 20×10^6 cells per mL.
- 22 Spin down at 200g for five minutes at room temperature.
- 23 Aspirate the supernatant and freeze by submerging the epi-tubes completely in liquid nitrogen.
- 24 After frozen (at least thirty seconds), remove from the liquid nitrogen and store at -80°C .