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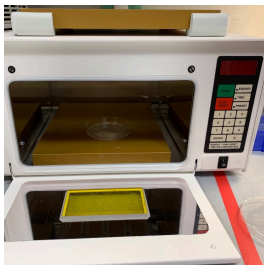
UV Crosslinking of Adherent Cells for eCLIP



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Protocol status: Working
We use this protocol and it's working

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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 adherent cells to prepare them for eCLIP experiments.

Materials

Required materials:

1. UV crosslinker with 254-nmwavelength 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)

Troubleshooting

Before start

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

1 Aspirate spent media

2 Wash the plate gently with 1x DPBS at room temperature (15 mL for a 15 cm plate).

3 Aspirate media

4 Add enough 1x DPBS to just cover the plate (5 mL for a 15 cm plate)

5 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

6 Place the tissue culture plate on leveled ice or a cooling block pre-chilled to 4°C

7 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.

8 Cross-link at 254-nm UV with an energy setting of 400 mJoules/cm^2
- Note: this is a setting of 4000 on many cross-linkers which display values in 0.1 mJoules/cm^2

9 While keeping the cells on ice, use a cell scraper (Corning, CLS3010-10EA) to scrape the plate.

10 Transfer the cells to a 50 mL conical tube

11 Wash plate once with 10mL of 1x DPBS and add to the same 50 mL tube.

12 Gently resuspend until the sample is homogeneous

13 Count cell concentration (either with automated cell counter or hemocytometer)
- **Note**: ensure cells are re-suspended well before counting
- **Note**: for cells that do not easily dissociate into single cells, a separate plate of cells can be counted instead (see * above)

14 Centrifuge the 50 ml conical tube at 200g for five minutes at room temperature.

15 Aspirate and discard supernatant.

16 Resuspend in the desired amount for flash freezing
- Typically 20×10^6 cells per mL.

17 Transfer desired amount into 1.5 mL Eppendorf Safe-Lock Tubes (or equivalent)
- Typically 1mL of 20×10^6 cells per mL.

18 Spin down at 200g for five minutes at room temperature.

19 Aspirate the supernatant and freeze by submerging the epi-tubes completely in liquid nitrogen.

20 After frozen (at least thirty seconds), remove from the liquid nitrogen and store at -80°C.