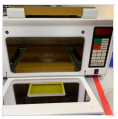

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UV Crosslinking of Suspension Cells for eCLIP

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1 Works for me dx.doi.org/10.17504/protocols.io.z62f9ge

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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.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Van Nostrand EL, Pratt GA, Shishkin AA, Gelboin-Burkhart C, Fang MY, Sundararaman B, Blue SM, Nguyen TB, Surka C, Elkins K, Stanton R, Rigo F, Guttman M, Yeo GW. Robust transcriptome-wide discovery of RNA-binding protein binding sites with enhanced CLIP (eCLIP). Nature Methods. 2016 Jun;13(6):508-14. PMID: 27018577. and Van Nostrand EL*, Nguyen TB*, Gelboin-Burkhart C, Wang R, Blue SM, Pratt GA, Louie AL, Yeo GW. Robust, Cost-Effective Profiling of RNA Binding Protein Targets with Single-end Enhanced Crosslinking and Immunoprecipitation (seCLIP). Methods Mol Biol. 2017;1648:177-200. PMID: 28766298. (*Joint first authors)

MATERIALS TEXT

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)

BEFORE STARTING

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

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

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 20×10^6 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.