

1.1 Adherent pulse chase

Seed cells in 35-mm dishes

Transfect/infect cells

Starve cells

Pulse cells

Wash cells 1x with chase solution then incubate in chase solution

Aspirate chase solution, transfer to ice and add stop solution

Wash 1x with stop solution

Lyse cells with scraping

Pellet nuclei

1.2 Suspension pulse chase

Culture cells

Transfect/infect cells

Starve cells

Pulse cells

Add 4x volume chase solution

Take 0 minute chase time

Take all other chase times

Pellet cells and wash 1x with stop solution

Lyse cells on ice

Pellet nuclei

2.0 Immunoprecipitation

Preincubate antibody + beads >30 minutes

Wash 2x 10 minutes with shaking

Resuspend beads in 20 μ l TE pH 6.8, add 20 μ l 2x sample buffer and heat

Add lysate

Reduce half of sample (if necessary)

3.0 SDS-PAGE

Prepare resolving and stacking gel

Load sample onto gel and run

Stain gel with staining solution

Destain gel

Dry gel using gel dryer