Isolation of nuclei from tissue culture cells

Risk assessment

– Work with human-derived material or transgenic cell lines (BSL-2)

▷ Wear gloves, safety glasses, lab coat

◻ Dispose waste after inactivation as REGULATED MEDICAL WASTE



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This is a bench card. Full protocol available online.

Preparation of cells

(1.) Start with 1×10^{6}–1×10^{9} cells. When working with frozen cell pellets, thaw on ice. Approximate the packed cell volume; this is 1 vol. Typically, 1×10^{9} cells amount to 1–2 mL cell volume.

> ***Critical:*** Keep buffer volumes to a minimum to maintain high protein concentrations.

(2.) Wash cells twice with 5 vol ice-cold PBS with inhibitors. Try to obtain a suspension free of visible clumps. Collect the cells at 400 × *g* for 5 min at 4 °C. 10 min

A › ### *Detergent-free isolation of nuclei*

(1.) Resuspend the cell pellet in 5 vol ice-cold 1 × hypotonic swelling buffer with inhibitors. Ensure the suspension is free of clumps. Incubate for 10 min on ice to swell the cells. 10 min

(2.) Collect the cells by centrifugation at 400 × *g* for 10 min at 4 °C, discard the supernatant. 10 min

(3.) Resuspend in 2 vol (at least 0.4 mL) swelling buffer. Transfer to a pre-chilled Dounce homogenizer.

> ***Critical:*** Do not vortex! Great care must be exercised throughout this procedure. Repeated pipetting and centrifugation will result in compromised nuclei which are very susceptible to leaking and disruption.

(4.) Using a type A (or “loose”) pestle, lyse the cells by 10–15 strokes. Apply even pressure and deliberate strokes for uniform lysis. Avoid the formation of bubbles and foam. Repeat until lysis is complete.

> ***Quality assurance:*** Monitor lysis by removing 5–10 µL lysate. Mix with an equal volume of 0.4% Trypan blue; view under a microscope. Stop homogenization when 95% of cells lost their cytoplasmic membrane and nuclei still remain intact.

(5.) Transfer to a centrifuge tube, pellet broken cells and nuclei at 400 × *g* for 5 min at 4 °C. Keep the supernatant as cytoplasmic fraction if desired.

> ***Quality assurance:*** The nuclear pellet should be white as opposed to the off-white/beige cellular pellet.

(6.) Wash once in 1.5 vol hypotonic swelling buffer to remove cell membranes.

(7.) Resuspend the nuclei in the desired buffer for downstream experiments.

B › *Isolation of nuclei in isosmotic sucrose*

(1.) Wash the cell pellet twice in 2–5 vol ice-cold isosmotic sucrose buffer with inhibitors.

(2.) Resuspend in 1–2 vol ice-cold sucrose buffer with inhibitors. Ensure the suspension is free of clumps. Admix an equal volume of sucrose buffer containing 0.6% Triton X-100. Incubate for 10 min on ice. 10 min

> ***Critical:*** If no single cell suspension is obtained at this point, cell lysis will not be homogenous. Avoid shearing membranes and organelles by pipetting very gently.

(3.) Pellet at 400 × *g* for 5 min at 4 °C. If desired, keep the supernatant as cytoplasmic fraction.

> ***Quality assurance:*** The nuclear pellet should be white as opposed to the off-white/beige cellular pellet.

(4.) Wash twice with 2–5 vol sucrose buffer to remove cell membranes.

(5.) Resuspend the nuclei in the desired buffer for downstream experiments.

Storage of isolated nuclei

(1.) Resuspend the nuclei in isosmotic sucrose buffer; snap-freeze in liquid nitrogen or a dry ice–ethanol bath. Store at −80 °C for up to two years. Do not refreeze.

Recipe (available online) Troubleshooting (available online) Notes (available online)

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