Protocols

BioTechniques Rapid Dispatches

Cryogenic disruption of mammalian cells using the Retsch PM 100

Protocol for:

Improved methodology for the affinity isolation of human protein complexes expressed at near endogenous levels

Michal Domanski[1], Kelly Molloy[2], Hua Jiang[3], Brian T. Chait[2], Michael P. Rout[3], Torben Heick Jensen[1], and John LaCava[3*]

[1]Centre for mRNP Biogenesis and Metabolism, Department of Molecular Biology and Genetics, Aarhus University, C.F. Møllers Allé 3 (Building 1130), DK-8000 Aarhus C, DK, [2]Laboratory Mass Spectrometry and Gaseous Ion Chemistry, The Rockefeller University, 1230 York Avenue, New York, NY 10065, USA, and [3]Laboratory of Cellular and Structural Biology, The Rockefeller University, 1230 York Avenue, New York, NY 10065, USA

Legend

⇨*ATTENTION*
* *HINT*
REST

Procedure

Cell harvesting and cell beads preparation

1. Grow cells on thirty 150 cm^2 culture dishes to ~90% confluence.

* *HINT As few as ten plates, as above, will produce sufficient cell material for this protocol (~1 g wet cell weight [WCW] input); however, recoveries are best when more cell material is used. This is due to small losses of material on the jar and ball surfaces. By the method described, using these materials, up to ~0.5 g WCW may be lost. Recoveries from thirty plates (~3 g WCW) are ~2.5 g or more.*

2. Aspirate the growth media.
3. Scrape the cells in 5 ml of ice-cold PBS per plate.
4. Pool the cells into 50 ml tubes.
5. Spin the harvested cells down at ~900 - 1000 RCF (2000 rpm in a Beckman GH-3.8 rotor), 4°C for 5 min.
6. Resuspend the cells in total 50 ml of ice-cold PBS and transfer to one 50 ml tube.
7. Spin the cells down as in step 5, above
8. Resuspend the cell pellet in 5 mL of ice-cold PBS and transfer to a capped 5 or 10 ml Luer lock syringe.

* *HINT If using fewer plates, a smaller syringe may be desirable for maximum recovery of cell material.*

9. Place the syringe inside a 50 ml tube and spin down as above.

* *HINT To fit the syringe fully within the 50 ml tube, the finger grips may need to be removed, if present. Either purchase syringes without finger grips, or cut them off with scissors.*

10. Aspirate a supernatant, place a plunger and inject the cells directly to a 50 ml tube containing N_2.
11. Using a needle, poke holes in the tube cap (punched lid). This will allow the N_2 to be decanted after the tube is capped, without losing the cell beads.
12. Cap the tube with the punched lid. Remove remaining N_2 by inverting the tube. Cell beads are ready for cryogenic disruption.
a. This is good for short-term storage (1-2 days). For long-term storage replace the punched lid with a new airtight cap to prevent frost accumulation on the cell beads.

REST Cell beads may be stored at -80°C prior to grinding

Cryogenic disruption

⇨*ATTENTION All tools for grinding and manipulations should be pre-cooled with N_2. A small decanter will be required for pouring N_2 over the top of the closed grinding jar, for cooling between cycles during the first phase of grinding, and for adding N_2 within the grinding jar during the second phase of grinding. A homemade tool is depicted with the equipment.*

Grinding, Phase I

13. Pre-cool 50 ml grinding jar with three 20 mm grinding balls in a clean Styrofoam box containing N2.
14. Set appropriate counterbalance on the PM 100.
15. Place the cells beads inside the grinding jar.
16. Perform three cycles of grinding using following program: 400 rpm, 3 min, reverse-rotation each minute, no interval break. Cool the grinding jar in N2 in between each cycle.

⇨*ATTENTION During grinding a distinct clunking noise is generated as the balls*

collide within the jar in planetary motion. If these sounds are not heard, grinding is not occurring and something has gone wrong. By the end of these cycles the cell material will already appear as a powder (grindate). In the event that a problem occurs and grinding is not achieved – e.g. cell beads remain – add N2 to the jar up to ~½ full and grind for an additional cycle, as above, or until no cell beads remain and then proceed to phase II (see note below step 18, below).

Grinding, Phase II

17. Replace the 20 mm grinding balls with eighteen N2 cooled 10 mm balls. 20 mm balls can be removed using large tweezers pre-cooled in N2. Before removing the 20 mm balls add N2 to the jar up to ~½ full – this will help recover cell material stuck on the surface of the balls.
18. Perform six cycles of grinding using following program: 400 rpm, 1 min. Fill the grinding jar with N2 up to ~½ volume in between each cycle.

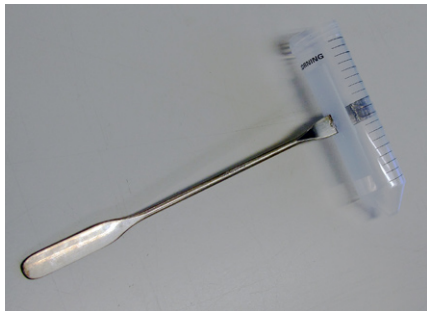
⇨ ***ATTENTION*** *The liquid N_2 added between each cycle will begin to evaporate within the jar as the temperature of the jar increases during grinding. This results in pressure build-up within the jar. When disengaging the jar from the grinder, one should release the clamp slowly. If the clamp is released rapidly, cell powder may escape with rapid depressurization. A slow release of the clamp allows the pressure to escape from the jar in a slow, controlled fashion, and prevents loss of cell material. The gentle escape of gaseous N_2 can often be heard as the clamp is slowly released – this is normal.*

19. Remove grinding balls and transfer the grindate to an N_2 cooled 50 ml tube using an N_2 cooled metal spatula.

⇨ ***ATTENTION*** *Cell grindate should be held at -80°C until use. In our experience, cell powder can be stored in this way, essentially indefinitely, without affecting performance.*

Equipment

Nunc 150 cm^2 culture dishes – cat. #168381 (http://www.nuncbrand.com/us/default.aspx)
50 ml tubes
5 or 10 ml Luer lock syringe
Syringe cap
Refrigerated centrifuge
Styrofoam box
RETSCH equipment (http://www.retsch.com/)
Planetary ball mill PM 100 – cat. #100 20.540.0001
Stainless steel grinding balls 10 mm Ø – cat. #05.368.0063
Stainless steel grinding balls 20 mm Ø – cat. #05.368.0062
Stainless steel grinding jar 50 ml – cat. #01.462.0149
Picture of homemade N_2 decanter (below)



Reagents

Growth media
Liquid nitrogen (N2)
Phosphate buffered saline (PBS)

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Address correspondence to John LaCava, Laboratory of Cellular and Structural Biology, The Rockefeller University, 1230 York Avenue, New York, NY 10065, USA. e-mail: jlacava@rockefeller.edu