InVivo Biosystems

Instructions for Using Large Scale Synchronized Worm Beads

Application 1. For protein purification, coimmunoprecipitation (CoIP), chromatin-IP (ChIP), chromatography, and related applications

Materials and equipment:

- Lysis buffer pre-chilled to 4ºC.
- Protease inhibitorsIce and ice bucket.
- Tissue homogenizing instrument, e.g. probe sonicator, Dounce homogenizer, bead mill, etc.
- Refrigerated centrifuge

Procedure:

1. Pre-chill all buffers and containers.
2. Remove Large Scale Synchronized Worm Beads from -80ºC. Keep on ice.
3. Immediately immerse beads in 1 or more volumes of lysis buffer with protease inhibitors.
4. Thaw quickly and resuspend thoroughly.
5. Lyse worms using probe sonication or your choice of tissue disruption method.*
6. Transfer to ultracentrifuge tube and centrifuge at >25,000g for 10min. (Alternatively, centrifuge at 15,000g in benchtop centrifuge.)
7. Transfer supernatant to a clean, pre-chilled container for downstream use.

**Evaluate lysis efficiency by collecting small samples of crude lysate, pellet, and supernatant and analyzing by SDS-PAGE.*

Application 2. For SDS-PAGE and Western Blotting

Materials and equipment:

- Laemmli sample loading buffer (2X or 4X)
- Ice and ice bucket.
- Benchtop centrifuge.
- Heat block or heat plateEquipment for SDS-PAGE
- Equipment for Western blotting (if desired)

Procedure:

1. Remove Large Scale Synchronized Worm Beads from -80ºC. Keep on ice.
2. Remove a single Worm Bead and return the rest to -80ºC.
3. Add Laemmli Sample buffer to 1X at 500µl (dilute or concentrate as needed).
4. Boil sample for 5min (or heat at 37ºC for 10min for sensitive applications).
5. Spin at maximum speed (~15,000 x g) in benchtop centrifuge.
6. Load 1-50µl of supernatant on separating gel.