**Purification of lysosomes using dextran-conjugated SPIONs**

Authors: Will Hancock-Cerutti1,2,3,4,5, Arun Kumar Tharkeshwar1, Shawn M. Ferguson1,5, Pietro De Camilli1,2,3,5

1Departments of Neuroscience and of Cell Biology, Yale University School of Medicine, New Haven, Connecticut 06510, USA

2Howard Hughes Medical Institute

3Program in Cellular Neuroscience, Neurodegeneration and Repair, Yale University School of Medicine, New Haven, Connecticut 06510, USA

4Interdisciplinary Neuroscience Program and MD-PhD Program, Yale University School of Medicine, New Haven, Connecticut 06510, USA

5Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, 20815

**Abstract**

This method describes the usage of dextran-conjugated superparamagnetic iron nanoparticles (SPIONs) to purify a lysosome fraction from cultured cells.

**Keywords:**

Dextran-conjugated superparamagnetic iron nanoparticles (SPIONs), lysosome purification, organelle purification, synthesis

**Purification of lysosomes with dextran-conjugated SPIONs**

**Solutions to prepare**

**1M HEPES buffer solution** (Gibco)

**DMEM** containing 10%FBS , 100U/ml penicillin, 100mg/mL streptomycin and 2mM L-glutamine (all from Gibco)

**Homogenization buffer (HB)**, containing 5mM Tris, 250 mM Sucrose, 1mM EGTA in mass-spectrometry grade water (ThermoFisher). Adjust pH to 7.4. Supplement with protease inhibitor cocktail (Roche) immediately before use.

**Prepared SPION solution** (See “Synthesis of colloidal dextran-conjugated superparamagnetic iron nanoparticles (SPIONs)” protocol**)**

**Protocol**

1. Plate HeLa-M cells on 4x 15 cm plates at 3.5 x 106 cells per dish.
2. The following day, exchange the the culture medium (DMEM) for fresh DMEM containing 10 mM HEPES and 10% SPION solution by volume. Incubate for 4 hours (pulse).
3. Change medium back to fresh DMEM and incubate for 15 hours (chase).

**NOTE**: It is not necessary to rinse the cells after removing the SPION containing media and rinsing will reduce lysosome yield

1. Rinse cells twice with PBS and then scrape into 5 mL of PBS on ice.
2. Centrifuge cells at 1000 rpm for 10 min at 4˚C.
3. Remove PBS and resuspended the cell pellet in 3mL HB (supplemented with protease inhibitor cocktail (Roche) immediately before use)
4. Pass cell suspension through a manual cell homogenizer (Isobiotec, 10 cycles, 10-micron clearance) to generate a total cell lysate.
5. Centrifuge the lysate at 800 g for 10 min at 4˚C to remove intact cells and cell debris.
6. During this 10 min centrifugation, set up magnetic LS column (Miltenyi Biotec) on magnetic rack at 4˚C and add 1 mL of HB to each column. Allow columns to drain.
7. Collect the supernatant and load onto pre-equilibrated magnetic LS column on magnetic rack. Allow sample to fully enter resin.
8. Wash the column with 5 mL HB. Allow HB to drain completely.
9. To elute, remove columns from the magnetic rack, place column into collecting tube and elute with 3 successive aliquots of 1mL HB forced through with positive pressure using the plunger provided with the column.

**OPTIONAL**: To increase lysosome yield, pipette 1 mL of the eluate back into the column and force through again. Repeat twice.

1. Centrifuge eluate at 55,000 rpm for 1 hr at 4˚C to pellet the lysosome fraction.
2. Remove supernatant and resuspend pellet in 200 µL of mass-spectrometry grade water (ThermoFisher). Flash freeze.