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# Endosome isolation

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**Protocol status:** Working
**We use this protocol and it's working**

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## Abstract

Subcellular fractionation to isolate early and late endosomes (EEs and LEs) by performing a series of centrifugation steps.

## Continuous sucrose gradient tube preparation

1 prepare 0.5M and 2M sucrose buffer

2 prepare buffer S2 by mixing 0.5M and 2M sucrose at 1:2 and buffer S3 at 2:1 ratio

3 place Beckman ultracentrifuge tube (No.344059) on dry ice

4 add 2.5ml 2M sucrose in the tube and wait for 00:10:00 or until it frozen. 10m

5 add 2.5 ml buffer S2 on top of frozen 2M sucrose and wait for 00:10:00 or until it frozen 10m

6 add 2.5 ml buffer S3 on top of frozen buffer S2 and wait for 00:10:00 or until it frozen 10m

7 add 2.5 ml 0.45M sucrose on top of frozen buffer S3 and wait for 00:10:00 or until it frozen 10m

8 keep the frozen tubes at -80 until experiment day

9 place the frozen tubes at 4C for 05:00:00 or until defrosted before use 5h

## Fractionation

10 Dissect striatum from mice, fresh freeze the tissue with dry ice

11 Prepare homogenization buffer (HB): 150mM Nacl, 10mM HEPES, 1mM EGTA, 0.1M MgCl

12 Homogenize striatum tissue with 1ml HB containing 1x Halt protease and phosphatase inhibitor (EDTA-free) by Dounce homogenization on ice (30 strokes)

13 Centrifugation 00:10:00 , 3,000g, 4C 10m

14 load postnuclear supernatants onto the top of continuous sucrose gradient

15 Centrifugation 18:00:00 , 200,000g, 4C (Accel and Decel → slow) 18h

16 Collect fractions from top to bottom of the gradient

## TCA precipitation 50m 3s

17 add equal volume of ice-cold 55% TCA and incubate on ice for 00:30:00 30m

18 centrifugation 00:10:00 , 20,000g, 4C 10m

19 wash pellet with 1ml ice-cold acetone centrifugation 00:10:00 , 20,000g, 4C 10m

20 go to step #19 for 2 times. Total 3 washes

21 resuspend pellet with 70ul of 1% SDS buffer (1% SDS, 10mMTris, 2mMEDTA) containing 1x Halt protease and phosphatase inhibitor (EDTA-free), sonication for 00:00:03 and followed by incubation on orbital shaker for 1hr at room temperature. 3s

22 Store samples at -80 for western blot assay