Organelle isolation from mouse tissues expressing organelle tags

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We use this protocol and it's working

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ABSTRACT

We describe here a method to perform the rapid isolation of intact lysosomes from mouse tissues expressing a lysosome-localized TMEM192-3×HA fusion protein ("LysoTag") (as in JAX stock #035401, RRID:IMSR_JAX:035401). The fusion protein marks lysosomes with a triple HA epitope, enabling the rapid isolation of intact tagged organelles from tissues using an anti-HA antibody. The organelles purified using this method are highly enriched, intact, largely contaminant-free and can be used for various downstream applications, including immunoblotting analysis and proteomic analysis (as described in dx.doi.org/10.17504/protocols.io.ewov1o627lr2/v1), but also lipidomic or metabolomic analysis (as described in dx.doi.org/10.17504/protocols.io.bybjpskn). ***This method can be adapted to isolate other tagged organelles from tissues.***

MATERIALS

Materials:

1. **Reagents**

Tissues collected from mice culled by cervical dislocation
Dulbecco's phosphate-buffered saline (PBS) (GIBCO. REF# 14190169)
KPBS Buffer: 136mM KCl, 10 mM KH2PO4 in Mass Spec grade water. Adjust to pH 7.25 with KOH.
"Supplemented KPBS" (to be prepared immediately before use): KPBS buffer supplemented with 1X phosSTOP phosphatase inhibitor cocktail (PhosSTOP tablet: Roche, REF# 04906837001) and 1X protease inhibitor cocktail (cOmplete EDTA-free protease inhibitor cocktail tablet: Roche, REF# 11873580001)
Thermo Scientific™ Pierce™ Anti-HA Magnetic Beads (Thermo Fisher Scientific, cat # 13474229)

1. **Equipment**

Belly Dancer Orbital Shaker (IBI Scientific, model # BDRAA115S)
DynaMag™-2 Magnet (Invitrogen. REF# 12321D)
2 ml Dounce homogeniser (VWR, cat no 89026-386; plunger cat no 89026-398)

1. **Consumables**

1.5 ml low binding Eppendorf tubes (Sarstedt. REF# 72.706.600).
Standard 1 ml and 200 µl Pipette tips (Greiner bio-one. Cat# 686271 and 685261 respectively).
Stripetter/stripette gun and stripettes
Set of Gilson pipettes P10, P200, P1000

1) Anti-HA Magnetic beads preparation

1 1.1) Transfer n x 100 µl of anti-HA Magnetic Beads (where n = number of samples) into a low binding Eppendorf tube on ice.

2 1.2) Immobilize the beads by placing the tube into a Dyna-Mag tube holder for 30s.

3 1.3) Remove the supernatant using a pipette.

4 1.4) Gently resuspend the beads in 1ml of KPBS.

5 1.5) Repeat steps 1.2 to 1.4.

6 1.6) Immobilize the beads by placing the tube into a Dyna-Mag tube holder for 30s.

7 1.7) Remove the supernatant using a pipette.

8 1.8) Gently resuspend the beads from step 1.7 in n x 100 µl of KPBS (where n = number of samples) to make a 1:1 slurry.

9 1.9) Aliquot the washed beads from step 1.8 into fresh low-binding Eppendorf tubes (100 µl of

slurry for each sample).

10 1.10) Leave the tubes on ice until use (step 3.1).

2) Tissue homogenisation

11 2.1) Collect tissues from mice culled by cervical dislocation.
Note: We recommend collecting and processing tissues from one mouse at a time.

12 2.2) Rinse the tissues briefly in cold PBS.

13 2.3) Transfer the tissues to a Falcon tube containing cold PBS on ice.

14 ***Note: The following steps should be performed in a cold room.***
2.4) Transfer each tissue into a 2 ml Dounce homogeniser pre-washed with PBS.
- *Lung: Process the whole organ.*
- *Brain: Process one hemisphere.*
- *Other tissues might need optimisation.*

15 2.5) Add 1 ml of ice cold supplemented KPBS to each tissue sample.

16 2.6) Homogenise the tissues as follows:
- *Lung: homogenise with 25 strokes.*
- *Brain: homogenise with 25 strokes.*
- *Other tissues might need optimisation.*

Note: We recommend performing the first 5 strokes of lung homogenisation before adding the KPBS, as residual air in the lungs can make homogenisation difficult.

17 2.7) Transfer the homogenate from step 2.6 to a low-binding Eppendorf tube on ice using a P1000 pipette.
Note: The pipette tip can be cut to ensure retrieval of all material.

18 2.8) Centrifuge at 1000 g for 2 min at 4°C to remove debris.

19 2.9) Transfer the supernatant from Step 2.8 into a new low-binding Eppendorf tube on ice.

20 2.10) For each sample, transfer 25-50 µL to a new low binding Eppendorf (= **input**) and keep on ice.
Note:
- *Rinse the Dounce homogeniser with a generous amount of MilliQ water 3 times and cold PBS once between samples.*
- ***Proceed to organelle isolation as rapidly as possible after tissue homogenisation to avoid organelle degradation.***

3) Organelle isolation

21 3.1) Add the remaining homogenate from step 2.10 to the pre-washed HA-Magnetic beads (Step 1.10).

22 3.2) Mix gently by flicking the bottom of the tube. Disperse any clumps by gently pipetting up and down 5 times.

23 3.3) Incubate with agitation on a Belly Dancer orbital shaker for 5 minutes at 4°C.

24 ***The following steps should ideally be performed in a cold room. If not available, keep working on ice.***

3.4) Place the tubes from Step 3.3 on a magnetic tube holder for 30s to immobilise the beads.

25 3.5) Discard the supernatant (a sample can be collected and considered as a flow-through).

26 3.6) Resuspend the beads from Step 3.5 in 1 ml of supplemented KBPS.

27 3.7) Immobilise the beads by placing the tubes in a Dyna-Mag tube holder for 30s.

28 3.8) Discard the supernatant.

29 3.9) Repeat steps 3.6 to 3.8.

30 3.10) Resuspend the beads in 1 ml of supplemented KPBS and transfer to a new low binding Eppendorf tube on ice.

31 3.11) Place the tubes in a Dyna-Mag tube holder for 30s.

32 3.12) Discard the supernatant.

The **organelle IP beads** (from step 3.12) and the **input** (from step 2.10) can now be processed for either immunoblotting analysis or proteomic analysis (as described in dx.doi.org/10.17504/protocols.io.ewov1o627lr2/v1), or for lipidomic or metabolomic analysis (as described in dx.doi.org/10.17504/protocols.io.bybjpskn).
Note: If isolating organelles for subsequent lipidomic or metabolomic analysis, use KPBS (instead of supplemented KPBS) in steps 3.6-3.10.
The **input** (Step 2.10) and **organelle IP beads** (Step 3.12) samples can be stored directly at -80°C and processed for analysis at a later time point. This allows the collection and processing of tissues from all mice in the same experimental cohort before subsequent analysis.