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Protocol for Lysosomal Hexose Enrichment in SH-SY5Y Cells

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol outlines the enrichment and quantification of hexoses within lysosomes of SH-SY5Y cells. Cells are subjected to serum starvation and sucrose loading, followed by enzymatic hydrolysis using invertase.

Materials

- SH-SY5Y cells
- 15 cm cell culture dishes
- DMEM (with and without serum)
- Sucrose (Sigma)
- Invertase from *Saccharomyces cerevisiae* (Sigma)
- 1x DPBS



Procedure

3d

- 1 Seed 15×10^6 SH-SY5Y cells into 15 cm cell culture dishes.
- 2 Incubate cells for 3 days under fed conditions (with serum).
- 3 Wash cells twice with 1XDPBS
- 4 Replace medium with serum-free DMEM and incubate cells for 72 hours to induce serum starvation.

3d

Note

This is a general protocol for hexose enrichment of lysosomes . It can be applied to a variety of cell types depending on the specific experimental

- 5 At 48 hours of serum starvation, supplement the medium with sucrose to a final concentration of 50 mM.
- 6 Continue incubation for an additional 24 hours (total of 72 hours under serum starvation).
- 7 After treatment, wash cells thoroughly with 1x DPBS to remove residual sucrose.
- 8 Incubate cells with 0.5 mg/ml invertase in serum-free DMEM for 1 hour at 37°C.

LysolP

- 9 After invertase treatment, proceed with lysosomal isolation using the LysolP protocol to isolate intact lysosomes.

Find protocol here: dx.doi.org/10.17504/protocols.io.bybjpskn



Note

In the final step of the LysolIP protocol, after three washes, proceed directly with hexose derivatization

Benzoyl-hexose derivatization

- 10 Perform benzoyl-hexose derivatization on the isolated lysosomal fractions for hexose analysis.
Find protocol here: dx.doi.org/10.17504/protocols.io.36wgqnxeygk5/v

Data acquisition and analysis of derivatized hexose/glucose

- 11 **Find protocol here:**
dx.doi.org/10.17504/protocols.io.81wgbw1xygpk/v1