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Cathepsin D assay to verify the retention of lysosomal content

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We use this protocol and it's working

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Disclaimer

Abstract

Cathepsin D assay is a fluorescence-based assay that leverage on the activity of cathepsin D, a lysosomal enzyme, to monitor the intactness of lysosome in the cell. Here, we describe a method where we used the measurement of cathepsin D activity to verify the intactness of lysosomes that were isolated from HEK293 cells based on anti-TMEM192 Lyso-IP. Our data showed an increase in the cathepsin D activity of lysosomal fraction when compared with whole cell fraction and Mock-IP fraction, an indication that the lysosomes are intact and viable.

Attachments

DOCX

Cathepsin D assay fo...

28KB

Materials

Materials:

1. Cell lines
- HEK293 **ATCC Catalog #**CRL-1573 , RRID:CVCL_0045)

2. Media and Reagents
- Cathepsin D Activity Assay Kit (Fluorometric) **Abcam Catalog #**ab65302

3. Equipment
- ClarioStar plate reader

4. Consumables
- FLUTAC flat bottom black 96-well plate **greiner bio-one Catalog #**655076
- Standard 1ml and 200µl Pipette tips (Greiner bio-one. Catalog# 686271 and 685261 respectively).

Troubleshooting

Seeding cells and performing Lyso-IP with anti-TMEM192 beads

1 Seed HEK293 cells in 15cm plates and allow to reach 80-90% confluency.

2 Perform Lyso-IP (using anti-TMEM192 beads) and Mock-IP (using BSA coated beads) as previously described in **dx.doi.org/10.17504/protocols.io.x54v9yp51g3e/v1**

Preparing sample for Cathepsin D assay

3 Add 2 µg of protein from Lyso-IP and whole cell lysate into the wells FLUOTAC flat bottom black 96-well plate. This should be done in duplicate.

4 Top up to 50 µL with lysis buffer provided in the kit.

> Note
>
> Due to little or no protein in the Mock-IP, use equal volume as Lyso-IP sample.

5 Prepare Blank sample in duplicate. This should contain only lysis buffer.

6 Prepare a reaction master mix for 9 wells:

- 450 µL reaction buffer (from the kit) per well.
- 18 µL substrate (from the kit)

> Note
>
> - Although there are 8 wells to be used, however make master mix for 9 wells to account for pipetting error.
> - If performing assays for more samples/replicates, adjust master mix accordingly.

7 Add 52 µL of master mix into each well. Gently mix but avoid bubbles. Cover plate with foil to avoid light exposure.

Plate reading and analysis. 2h

8 Start the ClarioStar plate reading machine and initiate the software.

9 Set temperature to 37 °C .

10 Set reading time to 00:05:00 for 24 cycles. This is total reading time of 02:00:00 . 2h

11 Set reading wavelength to Ex/Em = 328 µL / 460 µL .

12 Highlight and name the virtual wells, ensuring they correspond with the orientation of the plate.

13 Set direction of plate reading.

14 Open the plate holder and insert plate. Remember to remove foil covering before inserting the plate into the equipment.

15 Close plate holder and run the program.

16 After the completion of the run, export data in excel format and analyse it using GraphPad Prism.

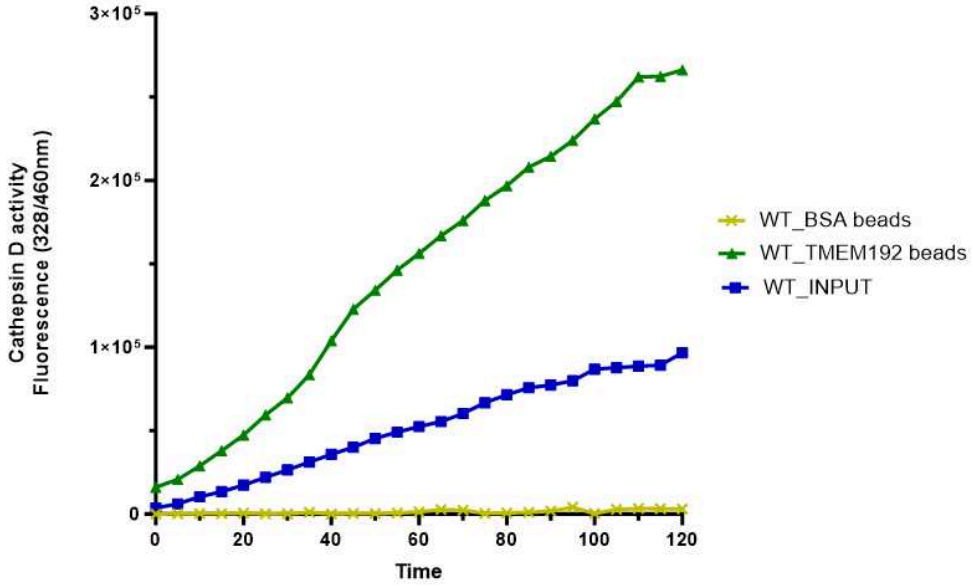

Figure 1: Cathepsin D activity showed that the purified lysosomes are intact and retain their content. After IP, Measure Cathepsin D activity from 2ug of protein obtained from lysosomal fraction and whole cell fraction while the Mock-IP serves as negative control. N=2