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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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## 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



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](https://doi.org/10.17504/protocols.io.x54v9yp51g3e/v1)

## Preparing sample for Cathepsin D assay

- 3 Add  2  $\mu\text{g}$  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  $\mu\text{L}$  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  $\mu\text{L}$  reaction buffer (from the kit) per well.
  -  18  $\mu\text{L}$  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  $\mu\text{L}$  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  $^{\circ}\text{C}$  .
- 10 Set reading time to  00:05:00 for 24 cycles. This is total reading time of  02:00:00 .
- 11 Set reading wavelength to Ex/Em =  328  $\mu\text{L}$  /  460  $\mu\text{L}$  .
- 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.

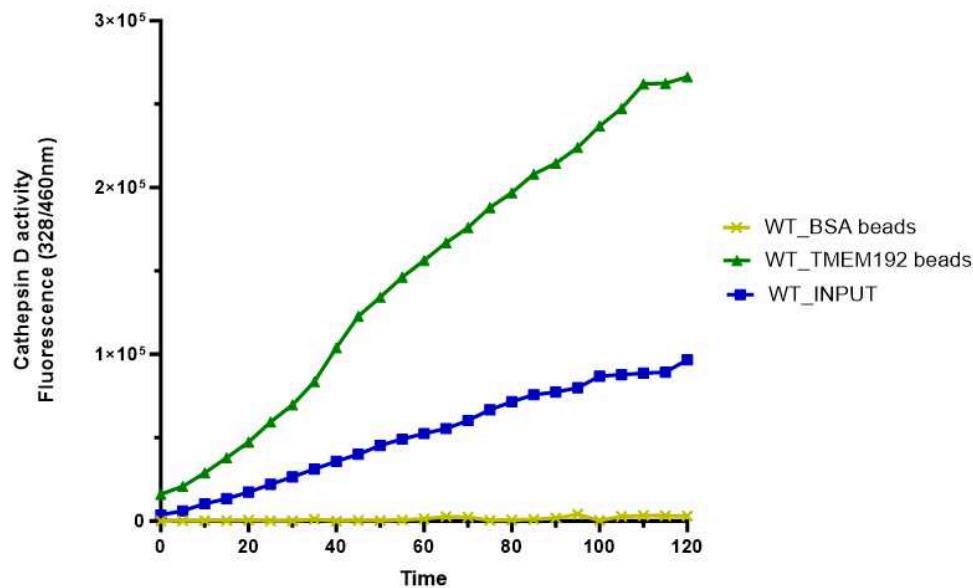


Figure 1: Cathepsin D activity showed that the purified lysosomes are intact and retain their content. After IP, Measure Cathepsin D activity from 2ug of protein obtained from lysosomal fraction and whole cell fraction while the Mock-IP serves as negative control. N=2