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Immuno-isolation of endogenously tagged BLTP3A^V5

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

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Abstract

This protocol explains how to immuno-isolate BLTP3A (BLTP3A^V5) from endogenously edited A549 cells

Guidelines

This protocol is designed to use the following cells lines: A549 wild-type (RRID:CVCL_0023); A549 BLTP3A^V5, B6 clone (RRID:CVCL_E9W3); A549 BLTP3A^V5, C5 clone (RRID:CVCL_E9W4)

Materials

15 cm plates (Corning 353025)
10 cm plates (Corning 430293)
dPBS (Sigma-Aldrich D8537)
anti-V5 resin (ChromoTek v5tma)
Trypsin (Gibco 25300054)
Eppendorf tubes (Fischer 05-408-138)
Cell scrapper (Fisher 353086)
Magnetic rack (Fischer 12321D)

Lysis buffer:
25 mM Tris pH 7.4 (Millipore Sigma T1503)
150 mM NaCl (Millipore Sigma S5886)
1% Triton (American Bioanalytical ab02025)
Protease inhibitors (Sigma-Aldrich 05056489001)

Complete media for A549 cells:
DMEM (Gibco 11965118)
10% FBS (Gibco/LifeTech 16140-071)
Pen-Strep (Gibco/LifeTech 15140-122)
Plasmocin (Invivogen ant-mpp)

Expanding cells

1 From one or more 10 cm plates at ~80-90% confluency, wash once with PBS before trypsinizing cells at 37C for 3-5 mins

2 Collect cells lifted off of the dish using complete media into a 15 mL Falcon tube 4m

3 Count the number of cells per mL of media using a hemocytometer or your choice of counting method 5m

4 Plate 1.5E6 cells onto to two 15 cm plates per condition per replicate: A549 wild-type and BLTP3A^V5 KI, in triplicate; for a total of 12 × 15 cm plates (six wild-type and six knock-in) 10m

5 Let the cells grow until ~90% confluent (typically 3-4 days) 4d

Lysate and anti-V5 preparation 3h 17m

6 Remove media from 15 cm plate and wash with at least 10 mL dPBS and then remove 5m

7 At 1 mL dPBS to each 15 cm plate (it works best to work with only two plates at a time to avoid cells drying out) 1m

8 Using a cell scrapper, scrape up as many cells as you can 5m

9 Pipette up as many cells as you can using the 1 mL of dPBS added to the dish and collect 2 × 15 cm plates in a single 2 mL Eppendorf tube on ice 5m

10 Spin down cells at 500g for 5 min in a refrigerated (4C) table top centrifuge 5m

11 Remove supernatant and resuspend cells in ice cold 1 mL lysis buffer 1m

12 Let cells rotate at 4C for 30 mins 30m

12.1 While cells rotate, prepare anti-V5 resin: add 75 uL resin to 1 mL ice cold lysis buffer in a new 2 mL eppendorf tube, invert 3 times, place on magnetic rack; leave at 4C until ready 5m

13 Spin down lysate at 17000g for 10 mins in a refrigerated (4C) table top centrifuge 10m

14 Collect supernatant in a new, ice cold 2 mL eppendorf tube 5m

15 Remove lysis buffer from resin and add lysate to resin 5m

16 Let lysate rotate at 4C with resin for 2 hours 2h

Resin washing and storage 22m

17 Place tubes on magnetic rack at 4C for 1 min to capture resin 1m

18 Remove supernatant and replace with 1 mL ice cold lysis buffer 5m

19 Rotate resin for 5 min at 4C 5m

20 Repeat this wash one more time (total of 2 washes) 10m

21 After final wash, place tubes on magnetic rack at 4C for 1 min to capture resin and then remove supernatant 1m

22 Spin down tube at 500g for 2 mins to pellet all buffer 2m

23 Put tubes back on magnetic rack at 4C for 1 min to capture resin 1m

24 Using a 200 uL pipette, remove the last little bit of buffer from the bottom of the tubes while still on the magnetic rack 5m

25 Store resin in absence of buffer at -80C