1

Mar 19, 2022

Detection of recombinant and endogenous LPPR3 by western blot V.1

cristina.kroon [1]

[1]Charité Universitätsmedizin Berlin

1

protocol .

cristina.kroon

This is a protocol for detection of overexpressed and endogenous LPPR3 from N1E-115 cells and primary hippocampal neurons.

cristina.kroon 2022. Detection of recombinant and endogenous LPPR3 by western blot. **protocols.io** https://protocols.io/view/detection-of-recombinant-and-endogenous-lppr3-by-w-b6grrbv6

protocol ,

Mar 19, 2022

Mar 19, 2022

59633

Item	Source	Catalog Nr	Lot nr	RRID
Transfer membrane, nitrocellulose	Carl Roth	HP40.1	NA	
Ponceau S solution	PanReach AppliChem ITW Reagents	A2935,0500	0J011658	
Powdered milk	Carl Roth	T145.2	371306718	
Goat-anti-rabbit IgG antibody (H+L) peroxidase	Vector Laboratories	PI-1000-1	ZG1009	AB_2336198
ECL Western Blotting Substrate	Promega	W1001	0000360871	

:

Sample preparation

1 N1E cells for detection of recombinant LPPR3

DIV 0: N1E cells were plated at a density of 150 000 cells/well (6-well plates) and grown overnight in DMEM medium (Gibco) with 10% FCS and 1% penicillin/streptomycin.
DIV 1: The cells were transfected with 1 µg of DNA using Lipofectamine 2000 (ThermoFisher Scientific) according to manufacturer´s protocol. The cells were grown overnight.
DIV 2: The cells were harvested.

Primary hippocampal neurons for detection of endogenous LPPR3

DIV 0: Cells were plated at a density of 500 000/well (6-well plates) and grown in Neurobasal A medium (Gibco) supplemented with 2% B27, 1% Glutamax and 1% penicillin/streptomycin for 9

days.
DIV 9: The cells were harvested.

Cell lysis

Lysis buffer:
Ripa buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.5% sodium deoxycholate, 1% NP40, 0.1% sodium dodecyl sulphate (SDS))
phosphatase inhibitors (1 mM Na_2MO_4,1 mM NaF, 20 mM β-glycerophosphate, 1 mM Na_3VO_4, 500 nM cantharidin)
protease inhibitors (Calbiochem set III, dilution 1:100)

All steps were carried out on ice. Cell culture medium was aspirated and the cells were quickly washed with ice cold PBS. 200 µl (neurons) or 300 µl (N1E cells) lysis buffer was added to each well, the cells were scraped off and collected. The homogenate was rotated at 4 degrees for 20 minutes and then centrifuged at 14 000 rpm at 4 degrees for 20 minutes. The supernatant was collected into a fresh Eppendorf tube.

Protein quantification

Protein quantification was carried out using the BCA Thermo Scientific Pierce™ Protein Assay according to manufacturers protocol.

WB sample preparation

50 µl of sample was mixed with 50 µl 4x Roti Load sample buffer and boiled at 95 degrees for 5 minutes.

SDS-PAGE and transfer

2 Protein samples (20 µg of total protein) were separated on a 8% gel at 80 V for 20 min and then at 120 V until the running front dye had ran out.

The proteins were transferred onto a nitrocellulose membrane for 2,5 hours at 400 mA (on ice to keep it from overheating).

Immunodetection

3 The membrane was rinsed in dH2O and stained with Ponceau S total protein stain for 3 minutes, after which the membrane was washed in dH2O for 2x1 min.

The blot was cut vertically to only include relevant lanes and the membrane was blocked in 5% milk in TBS-T for 1 hour at room temperature.

The membrane was incubated with primary antibody against LPPR3/PRG2 (1:1000, house-made, Brosig & Fuchs et al 2019) in 5% milk in TBS-T overnight at 4 degrees.

The blot was washed 3 times for 10 minutes in TBS-T.

The blot was incubated with anti-rabbit-HRP in 5% milk in TBS-T for 1 hour at room temperature.

The blot was washed 3 x 10 min in TBS-T.

Signal detection

4 The signal was detected with ECL Kit according to manufacturer´s protocol.

The blot was incubated in 500 µl ECL reaction for 1 min and the blot was imaged using Fusion SL camera (VilberLourmat, Germany) and manufacturer´s software. The blot was imaged in auto-exposure mode with final exposure time of 3 minutes and 25 seconds. Molecular weight marker and chemiluminescent signal images were automatically overlain by the software creating the image shown as blot source data.

Materials

5

Item	Source	Catalog Nr	Lot nr	RRID
Transfer membrane, nitrocellulose	Carl Roth	HP40.1	NA	
Ponceau S solution	PanReach AppliChem ITW Reagents	A2935,0500	0J011658	
Powdered milk	Carl Roth	T145.2	371306718	
Goat-anti-rabbit IgG antibody (H+L) peroxidase	Vector Laboratories	PI-1000-1	ZG1009	AB_2336198
ECL Western Blotting Substrate	Promega	W1001	0000360871	