

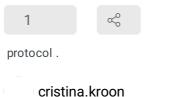


Mar 19, 2022

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

#### cristina.kroon 1

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

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https://protocols.io/view/detection-of-recombinant-and-endogenous-lppr3-by-wb6arrbv6

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

:

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#### 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  $\mu$ g 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  $\beta$ -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  $\mu$ l (neurons) or 300  $\mu$ l (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

 $50 \mu l$  of sample was mixed with  $50 \mu l$  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  $\mu$ l 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

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Item	Source	Catalog Nr	Lot nr	RRID
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peroxidase				
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