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Western blot - TBK1 (Cell Signaling, #51872S) cycloheximide assay

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Jailyn Izu¹

¹NIMH



Christopher Bartley

Translational Immunopsychiatry Unit, NIMH

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Protocol status: Working

We use this protocol and it's working

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Abstract

TBK1 immunoblot after cycloheximid assay

Materials

- 4-12% Bis-Tris (Invitrogen NuPAGE-SDS) mini gels (1.0 mm x 10 well or 1.5 mm x 15 well) (Invitrogen, #NP0321BOX /#NP0323BOX)
- MOPS-SDS running buffer (Invitrogen, #NP0001)
- 4x LDS buffer (Invitrogen, #NP0007)
- 10x dithiothreitol/DTT (Thermo Fisher Scientific, #A39255)
- Li-Cor Chameleon Duo Pre-stained Protein Ladder (Li-Cor, P/N: 928-60000)
- Tris Glycine transfer buffer 10X (KD Medical, #RGF-3391)
- HPLC Methanol (Fisher Scientific, #A452SK)
- Li-Cor Intercept (TBS) blocking buffer (Li-Cor, P/N: 927-60001)
- Tris-buffered saline 10X (Corning, #46-012-CM)
- Tween20 (Sigma, #P1379)
- Revert 700 Total Protein Stain for Western Blot Normalization (Li-COR Biosciences, #926-11010)
- **Antibodies: TBK1 (Cell Signaling, #51872S)**
- Sodium azide (Sigma, #S-8032)
- IRDye 800CW Goat anti-Mouse IgG Secondary Antibody (Li-Cor, P/N: 926-32210), IRDye 680RD Goat anti-Rabbit IgG Secondary Antibody (Li-Cor, P/N: 926-68071), IRDye 800CW Donkey anti-Chicken Secondary Antibody (Li-Cor, P/N: 926-32218)
- Immun-Blot LF PVDF Membrane (BioRad, #1620264)
- Filter papers (Whatman, #1001125)
- XCell II Blot Module (Invitrogen, #EI9051)
- 1.5 mL eppendorf tubes
- Transfer sponges
- Aluminum foil
- Li-COR Odyssey M



Western blot

- 1 Follow the general western blot protocol:

Protocol

NAME

General western blot protocol

CREATED BY

Jailyn Izu

Preview

Protocol-specific parameters:

- For total protein loading amounts in **Step 3**, load  10-20 μg of total protein for each sample (calculate from Bradford Assay)
- For the TBK1 primary antibody (Cell Signaling, #51872S) in **Step 14**, use a 1:1000 dilution (v/v) in blocking buffer: 6 mL Licor blocking buffer solution + 6 μl Tween20 (0.1%) +  6 μL TBK1 primary antibody