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Western blot - HA (Cell Signaling, #51872S) and anti-TBK1 to distinguish endogenous vs overexpressed TBK1

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Jailyn Izu[1]

[1]NIMH

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

Western blot to distinguish endogenous vs overexpressed TBK1

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)
- Sodium azide (Sigma, #S-8032)
- **HA primary antibody (Cell Signaling, #3724S)**
- **TBK1 primary antibody (Cell Signaling, #51872S)**
- **Beta actin antibody (Cell Signaling, #4970S)**
- **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)**
- 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

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Jailyn Izu

Preview

Protocol-specific parameters:

- For **Step 3**, load 3 uL lysate
- Skip **Steps 11-12** (normalizing to beta-actin instead of total protein)
- *Note*: **before Step 14**, you will cut the membrane with the transferred proteins at the 50 kDa mark and probe the top (Gel 1/1) and bottoms (Gel 1/2) with separate primary and secondary antibodies.
- For Gel 1/1 for the HA primary antibody (Cell Signaling, #3724S) 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 HA primary antibody
- For Gel 1/1 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
- For Gel 1/2 for the beta actin antibody (Cell Signaling, #4970S) in **Step 14**, use a 1:6000 dilution (v/v) in blocking buffer: 6 mL Licor blocking buffer solution + 6 µl Tween20 (0.1%) + 1 µL beta actin primary antibody
- For **Step 17**, see materials for secondary antibodies