



Jun 04, 2025

Western blot - ERK1 (Santa Cruz Biotechnology Inc., #sc-271269) protein expression

DOI

<https://dx.doi.org/10.17504/protocols.io.e6nvw1jbwlmk/v1>

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Protocol Citation: Jailyn Izu 2025. Western blot - ERK1 (Santa Cruz Biotechnology Inc., #sc-271269) protein expression. protocols.io <https://dx.doi.org/10.17504/protocols.io.e6nvw1jbwlmk/v1>

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Protocol status: Working

We use this protocol and it's working



Created: May 15, 2024

Last Modified: March 26, 2026

Protocol Integer ID: 99862

Keywords: protein expression erk loading control blot, western blot, protein expression, erk1

Funders Acknowledgements:

National Institute of Mental Health

Grant ID: ZIA MH002982

National Institute of Mental Health

Grant ID: R01MH122471

National Institute of Neurological Disorders and Stroke

Grant ID: U01NS120836

Foundation for the National Institutes of Health

Grant ID: Deeda Blair Research Initiative for Disorders of the Brain

Abstract

ERK loading control blot

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)
- **Antibodies: ERK1 (Santa Cruz Biotechnology Inc., #sc-271269)**
- 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

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Preview

Protocol-specific parameters:

- For total protein loading amounts in **Step 3**, load  5 μg of total protein for each sample (calculate from Bradford Assay)
- Skip **Steps 11 & 12** (normalizing to ERK1 instead of total protein)
- For the ERK1 primary antibody (Santa Cruz Biotechnology Inc., #sc-271269) 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 ERK1 primary antibody