Western blot

Laura Ruiz Remolina

Abstract

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Guidelines

Avoid repeated freeze-thaw cycles

Before start

Prepare all the reagents and mind the pH of each one

Materials

- Phosphatase inhibitor PhosSTOP 04 906 837 by Roche
- Proteases inhibitor Complete 11 697 498 by Roche
- DC protein assay 5000112 by BIO-RAD
- BSA A7906 by Sigma Aldrich
- Acrilamide 161-0107 by BIO-RAD
- 2-mercaptoethanol M-6250 by Sigma-aldrich
- APS A-3678 by Sigma Aldrich
- Bisacrilamide 161-0201 by BIO-RAD
- H2O2 8597 by Merck Millipore
- Iodophenol I1020-1 by Sigma-aldrich
- Glicerol 4095 by Merck Millipore
- Glycine 161-0724 by BIO-RAD
- ✓ HCl 20248.295 by Contributed by users
- Luminol A-8511 by Sigma-aldrich
- ✓ PVDF membranes #66543 by Contributed by users
- ✓ Methanol 141091.1214 by Contributed by users

NaCl S-3014 by Sigma-aldrich

TEMED (Tetramethyl-ethulenediamine) T9281 by Sigma-aldrich

SDS 161-0302 by BIO-RAD

Tween-20 P-7949 by Sigma-aldrich

Protocol

• Preparation of lysate from tissue Remove a small volume of lysate to perform a protein quantification assay (with DC protein assay, BIO-RAD). Determine the protein concentration for each tissue lysate.

Step 1.

Load equal amounts of protein into the wells of the SDS -PAGE gel, along with molecular weight marker 60 ug total proteins per pore Run the gel for 90min at 100 V Gel percentage separation gel 10% spacer gel 5%.

Step 2.

Activate PVDF with methanol for 1 min and rinse with transfer buffer before preparing the stack Run at 100 V for 90min.

Step 3.

• Wash the membrane in three washes of TBST 5 percent skimmed milk powder 5 min each

Step 4.

Block the membrane 4 hours in BSA 5%. Wash the membrane in 3 washes of TBST, 5 min each.

Step 5.

Wash 3 times, 5 minutes each

Step 6.

Incubate the membrane with the primary antibody diluted as the manufacter’s instructions.

Step 7.

Wash 3 times, 5 minutes each

Step 8.

Incubate the membrane with the recommended dilution of conjugated secondary antibody in blocking buffer at room temperature for 1 h

Step 9.

. Wash the membrane in 3 washes of TBST, 5 min each

Step 10.

Acquire image using normal image scanning methods for colorimetric detection.

Step 11.