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🌐 immunoblotting (JMS protocol)

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Aug 05, 2021

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dx.doi.org/10.17504/protocols.io.bthcnj2w

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ABSTRACT

imonoblotting protocol JMS

1
determination of protein content by a bicinchoninic acid (BCA) assay

2
cell extracts containing 15 µg of total protein were separated on 4-15% sodium dodecyl sulfate-polyacrylamide gradient (150V, Bio-Rad) gels

3
electroblot gel onto polyvinylidene difluoride membrane

4
incubate membranes with [M]**0.4 % volume** paraformaldehyde for ⏱ **00:15:00** for better protein binding

15m

5
Block membranes with [M]**5 Mass Percent** milk for ⏱ **00:30:00**

30m

6 incubate blot with primary antibody for ⏱ **04:00:00 or overnight at 4°C**   4h

7 wash blot for 3 times for ⏱ **00:05:00** with PBS- [M]**0.1 % volume** TRITON-X100   5m

8 incubate for ⏱ **01:00:00** with horseradish peroxidase-conjugated secondary antibody (Dako, Glostrup)   1h

9 detect using chemiluminescence