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immunoblotting (JMS protocol)

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ABSTRACT

imonoblotting protocol JMS

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PROTOCOL INTEGER ID

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- 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 | |