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Western blot

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MTS



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Protocol status: Working

We use this protocol and it's working



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Abstract

The protein levels of BRC tissue and MTSs was detected via western blot.

Materials

- Cold RIPA lysis buffer (Beyotime)
- BCA protein assay kit (Beyotime)
- 10% SDS-PAGE gels
- Polyvinylidene difluoride (PVDF) membranes (Merck)
- 5% skim milk
- HRP-conjugated secondary antibodies
- Fujifilm LAS 4000 luminescent image analyzer (Fujifilm Life Science)
- Quantity One software (Bio-Rad)
- Primary antibodies against PARP1, Bax, Actin, NF- κ B, p-NF- κ B, P38, p-P38, β -Catenin, full-length and cleaved caspase-3 (CST)
- Primary antibodies against GAPDH, Notch1, CD44, CD24, ALDH1A1, P53, PCNA, c-Myc, YAP (Proteintech Company)
- Goat anti-mouse or anti-rabbit HRP-conjugated secondary antibodies (Beyotime Company)

Western blot

- 1 Harvest and lyse tissues or sorted MTSs from MWC-chips in cold RIPA lysis buffer (Beyotime).
- 2 Centrifuge at 12,000 rpm for 15 min at 4°C.
- 3 Clarify lysates and collect supernatants for protein concentration determination using the BCA protein assay kit (Beyotime).
- 4 Fractionate proteins on 10% SDS-PAGE gels and transfer to polyvinylidene difluoride (PVDF) membranes (Merck).
- 5 Block PVDF membranes with 5% skim milk at room temperature for 1 hour.
- 6 Incubate overnight with primary antibodies.
- 7 Apply HRP-conjugated secondary antibodies to membranes for an additional hour.
- 8 Visualize blots using chemiluminescence and photograph using Fujifilm LAS 4000 luminescent image analyzer (Fujifilm Life Science).
- 9 Quantify grayscale values using Quantity One software (Bio-Rad).