

## NB21-1022 Protocol

### Western Blot protocol specific for H3K4me2 antibody (NB21-1022)

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Histone H3 [Dimethyl Lys4] Antibody: [https://www.novusbio.com/products/histone-h3-antibody\\_nb21-1022](https://www.novusbio.com/products/histone-h3-antibody_nb21-1022)

1. Perform SDS-PAGE on samples to be analyzed, loading 40 ug of total protein per lane.
2. Transfer proteins to membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain according to standard Ponceau S procedure (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot.
5. Block the membrane using standard blocking buffer for at least 1 hour.
6. Wash the membrane in wash buffer three times for 10 minutes each.
7. Dilute the primary antibody in blocking buffer and incubate 1 hour at room temperature.
8. Wash the membrane in wash buffer three times for 10 minutes each.
9. Apply diluted HRP-conjugated secondary antibody in blocking buffer (as per manufacturers instructions) and incubate 1 hour at room temperature.
10. Wash the blot in wash buffer three times for 10 minutes each (this step can be repeated as required to reduce background).
11. Apply the detection reagent of choice in accordance with the manufacturers instructions.