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## NB100-724 Protocol

# Western Blot Protocol for CTGF Antibody (NB100-724)

CTGF/CCN2 Antibody: https://www.novusbio.com/products/ctgf-ccn2-antibody_nb100-724
Western Blot Protocol

1. Perform SDS-PAGE (4-12%) on samples to be analyzed, loading 20 ug of total protein per lane.
2. Transfer proteins to Nitrocellulose according to the instructions provided by the manufacturer of the transfer apparatus.
3. Stain the blot using ponceau S for 1-2 minutes to access the transfer of proteins onto the nitrocellulose membrane. Rinse the blot in water to remove excess stain and mark the lane locations and locations of molecular weight markers using a pencil.
4. Rinse the blot in TBS for approximately 5 minutes.
5. Block the membrane using 5% non-fat dry milk + 1% BSA in TBS for 1 hour at RT.
6. Rinse membrane once in TBS and then wash 3x 10 minutes.
7. Dilute the rabbit anti-CTGF primary antibody (NB 100-724) in blocking buffer and incubate 1 hour at room temperature.
8. Rinse membrane once in TBS and then wash 3x 10 minutes.
9. Apply the diluted rabbit-IgG HRP-conjugated secondary antibody in blocking buffer (as per manufacturers instructions) and incubate 1 hour at room temperature.
10. Rinse membrane once in TBS and then wash 3x 10 minutes.
11. Apply the detection reagent of choice in accordance with the manufacturers instructions (Pierce's ECL is the standard reagent used at for this Novus Biologicals assay).

Note: Tween-20 can be added to the blocking buffer at a final concentration of 0.05-0.2%, provided it does not interfere with antibody-antigen binding.