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## NBP2-42215 Protocol

# Western Blot Protocol for BDNF Antibody (NBP2-42215)

Reagents needed:
a. Washing Buffer: Tris Buffer Saline with 0.01% of tween 20).
b. Blocking Buffer: 5% skimmed milk powder in washing buffer).
c. Secondary antibody, Horseradish peroxidase conjugated.
d. Chemiluminescent solution (SuperSignal WestPicoTM, Pierce).

Western blot Method:
1. Perform SDS-PAGE using PVDF membrane. Cut into strips.
2. Activate strips with methanol by dipping them into methanol for 5 min.
3. Discard the methanol and take fresh methanol to repeat step b.
4. Let the strips dry, and then add blocking solution and incubate at RT in a shaker for 30-45 minutes.
5. Dilute primary antibody in blocking buffer. Incubate the number of strips required with the diluted primary antibody at room temperature for 2 hours in a shaker.
6. Wash strips two times with washing buffer at 30 minutes intervals.
7. Dilute HRP conjugated secondary antibody in blocking buffer. Add diluted secondary antibody to the membrane strips and incubate for exactly 1 hour while shaking at RT.
8. Wash the strips with washing buffer for 2-3 hours with 3 to 4 changes on a shaker. This helps in reducing the back ground staining.
9. Prepare the chemiluminescent solution (SuperSignal WestPicoTM) by mixing solution A and Solution B at 1:1. Mix well. Soak the strip in the chemiluminescent solution; keep for 3-5 minutes under constant shaking.
10. Expose the membrane to a sheet of film and develop.