SynthImmunol_NMU Notebook No.1

Time: 2024.06.01-2024.06.03

---

1. **Experiment:** Western Blot
2. **Time:** 2024.06.01-2024.06.03
3. **Member:** Hanyue Liu, Xudong Tang, Yaqi Gao
4. **Material:** Protein extraction reagent (Thermo Scientific Pierce, 78505), BCA protein quantification kit (Thermo Scientific Pierce, A53225), loading buffer (5×, Beyotime, P0015), sample protein Marker (Invitrogen, 26625), membrane transfer system (Bio-Rad), 5% BSA (BBI, A600903), GPC3 antibody (abcam, ab95363), luciferase antibody (abcam, ab16466), β-actin antibody (abcam, ab179467), ECL luminescence detection reagent (Thermo Scientific Pierce, 32109), etc.
5. **Method:**
   (1) 5×10^6 cells of each cell line were taken, centrifuged at 3000 rpm for 5 mins, and transferred to 1.5 mL EP tube.
   (2) 200 μL protein extraction reagent (Thermo Scientific Pierce, 78505) was added to the cell precipitate and resuspended. Then, it was ice bathed for 30 mins, and oscillated every 5 mins.
   (3) After centrifugation at 13000 rpm for 20 mins at 4°C, the supernatant was carefully transferred to a clean EP tube, and the BCA protein quantification kit (Thermo Scientific Pierce, A53225) was used for protein quantification. After quantification, the protein concentration of each group was adjusted to be consistent, and 5× loading buffer (Beyotime, P0015) was added to the protein sample and mixed well. The mixture was then boiled in a water bath at 100°C for 5 mins, and the sample was loaded after cooling (10-20 μL protein/lane). At the same time, the sample protein Marker (Invitrogen, 26625) was loaded.
   (4) Proteins were separated by 12% SDS polyacrylamide gel electrophoresis.
   (5) The protein was transferred to the nitrocellulose membrane by a membrane transfer system (Bio-Rad).
   (6) At room temperature, TBST containing 5% BSA (BBI, A600903) was blocked for 2 hrs.
   (7) Elution with TBST twice, 20 mins each time.
   (8) GPC3 antibody (abcam, ab95363) was diluted at 1:500. Luciferase antibody (abcam, ab16466) was diluted at 1:500. β-actin antibody (abcam, ab179467) was diluted at 1:1000. These reagents were all incubated overnight at 4°C.
   (9) Elution with TBST three times, 15 mins each time. The horseradish peroxidase coupled second antibody was added and incubated at room temperature for 1-2 hrs.
   (10) Elution with TBST three times, 15 mins each time.
   (11) The solution A and solution B in the ECL luminescence detection reagent (Thermo Scientific Pierce, 32109) were mixed in equal volume, incubated at room temperature for 1-5 mins, and detected on the imaging analyzer.