Anti-Neu5Gc Antibody Kit Protocol - Western Blot V.3

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Abstract

The Anti-Neu5Gc Antibody Kit contains the essential monospecific polyclonal chicken IgY antibody, along with a negative control primary antibody to detect the presence of Neu5Gc on glycoconjugates by Western blot (WB). Samples to be evaluated are first subjected to SDS-PAGE, followed by transfer to a nitrocellulose or polyvinylidenedifluoride (PVDF) membrane. The membrane is then incubated with affinity-purified polyclonal anti-Neu5Gc to determine the presence of Neu5Gc on the protein of interest.

The antibody provided in this kit has been shown to identify as little as 5pmol of Neu5Gc per µg glycoprotein, which is at or below the current detection limit for conventional analysis by acid release, purification, DMB derivatization, HPLC, and electrospray mass-spectrometry. The Western blot provides additional information in that it confirms that Neu5Gc is directly linked to the glycoprotein of interest rather than to an accompanying sample component.

Materials

MATERIALS

Anti-Neu5Gc Antibody Kit **BioLegend Catalog #**146901

- Electrophoresis setup for SDS-PAGE and blotting
- Molecular weight marker
- Positive control protein
- Negative control
- TBS and TBS-T
- Enzyme-conjugated secondary anti-chicken IgY antibody (HRP or AP)

Troubleshooting

1 Prepare two identical SDS-PAGE gels.

2 Load and run samples in loading buffer.

3 Recommended loading of gel:
- **Lane 1:** Molecular Weight Marker
- **Lanes 2, 8, 10:** Blank
- **Lane 3-6:** Samples
- **Lane 7:** Positive Control
- **Lane 9:** Negative Control

4 Confirm the presence of proteins on gel by Coomassie Staining

5 Blot proteins to membrane of choice.

6 Confirm protein transfer by Ponceau or other method of choice.

7 Block each membrane in 20ml TBS-T with 200µl diluted Neu5Gc Assay Blocking Solution, gently rocking at 4°C.

8 Incubate first blot in Primary Antibody, and one blot in Control Antibody for 2 hours at 25°C or overnight at 4°C with gentle rocking.Note: Recommended range of dilution for Western Blot is 1:1,000 to 1:10,000. The final dilution of the Primary Antibody can vary with the material being probed and the amount of Neu5Gc present. The Control Antibody should be used at the same dilution as that used for the Primary Antibody.

9 Wash blot with 50ml TBS-T for 5 min at 25°C, gently rocking 5 times.

10 Incubate blots with optimal dilution of enzyme-conjugated secondary antibody of choice at 25°C for 1 hour.

11 Wash blot with 50 ml TBS-T for 5 min at 25°C, gently rocking 5 times.

12 Develop each blot with the appropriate substrate.