Anti-Neu5Gc Antibody Kit Protocol - Western Blot Version 2

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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 5 pmol of Neu5Gc per ug 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 Neu5G is directly linked to the glycoprotein of interest rather than to an accompanying sample component.

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Guidelines

Items required but not supplied:

- Electrophoresis setup for SDS-PAGE and blotting

- Molecular weight maker

- Positive control protein

- Negative control

- TBS and TBS-T

- Enzyme-conjugated secondary anti-chicken IgY antibody (HRP or AP)

Protocol

Step 1.

Prepare two identical SDS-PAGE gels

Step 2.

Load and run samples in loading buffer

Step 3.

Recommended loading of gel:

Lane 1: Molecular Weight Marker

Lanes 2, 8, 10: Blank

Lanes 3-6: Samples

Lane 7: Positive Control

Lane 9: Negative Control

Step 4.

Confirm the presence of proteins on gel by Coomassie Staining

Step 5.

Blot proteins to membrane of choice

Step 6.

Confirm protein transfer by Ponceau or other method of choice

Step 7.

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

Step 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

NOTES

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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.

Step 9.

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

Step 10.

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

DURATION

01:00:00

Step 11.

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

DURATION

00:05:00

Step 12.

Develop each blot with the appropriate substrate