

# Anti-Neu5Gc Antibody Kit Protocol - ELISA V.4

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## Abstract

This protocol is essentially the same as a standard ELISA protocol, with one major exception - all animal compounds must be avoided in buffers, etc. because they contain Neu5Gc, which will interfere with antibody activity and result in misleading data. Essentially, the glycoconjugate to be evaluated is immobilized on an ELISA plate and exposed to a primary antibody. The antibody provided in this kit has been shown to identify as little as 5pmol of Neu5Gc per microgram glycoprotein, which is at or below the current detection limit for conventional DMB HPLC analysis. An advantage of the ELISA procedure is that it confirms the presence of Neu5Gc on the glycoconjugate of interest, but use caution, as it also reacts with solution/media that contains glycoconjugate.

## Materials

- High-binding 96-well ELISA plate
- Sodium carbonate-bicarbonate immobilization buffer 50mM, pH 9.5
- Positive control target
- Negative control target
- PBS and PBS-T
- Enzyme-conjugated secondary anti-chicken IgY antibody (HRP or AP)
- Enzyme substrate



- 1 Coat the 96-well ELISA plate with the target molecule in 50mM sodium carbonate-bicarbonate buffer, pH 9.5 (up to 1µg/well in 100µl carbonate buffer, in triplicate) for at least 2 hours at ambient temperature or overnight at 4°C.
- 2 Wash the wells 3 to 5 times with PBS.
- 3 Block wells with PBS-T (200µl/well) for 1 hour at room temperature.
- 4 Add the anti-Neu5Gc antibody (or the negative control antibody), diluted in PBS-T, to the appropriate well(s) and incubate for 2 hours at room temperature.

Note: The resuspended primary and control antibody dilution range is between 1:1,000 and 1:10,000. The optimal dilution will need to be determined by the researcher. The suggested dilution for the secondary antibody is 1:5,000, but this should also be optimized for the particular application.

- 5 Wash wells as before with PBS-T.
- 6 Add enzyme-conjugated secondary antibody (donkey-anti-chicken antibody, 1:10,000 in PBS) to each well and incubate for 1 hour at room temperature.
- 7 Wash wells again with PBS-T.
- 8 Add enzyme substrate, allow the wells to develop; quench the reaction if necessary.
- 9 Read absorbance on an ELISA plate reader at the appropriate wavelength.