Chicken anti-Neu5Gc on frozen tissue

**Chicken anti-Neu5Gc IHC-HRP on frozen tissue**

**Date:**

**Goal:** To detect Neu5Gc in MOUSE and/or HUMAN tissues.

Washing Buffer: 1XPBST (pH7.1, with 0.1% Tween 20) Diluting Buffer: 0.5% Fish Gelatin in 1XPBST

**Materials:**

1. 10% buffered formalin: Fisher SF93-4
2. 30% H₂O₂: Fisher H325-100.
3. Avidin-Biotin blocking kit: Vector- SP2001
4. Sodium Periodate: Fisher S398-100
5. AUS (Arthrobacter Ureafaciens Sialidase): EY Labs Cat # EC-32118-5
6. 10% goat serum in 0.5% cold water fish gelatin/PBST as an inhibitor
7. Cold Water Fish Gelatin: Sigma G7765-250ML
8. Biotinylated donkey anti-Chicken IgY: Jackson Labs 703-065-155
9. HRP Streptavidin: Jackson labs 016-030-084
10. Mayer’s hematoxylin: Sigma MHS32
11. AEC substrate: Vector SK4200
12. Aqueous mounting media: aquamount: Vector H5501

**Slides to use:**

- a. + 0.5% fish gelatin
- b. Chicken IgY at 1:500 diluted in 0.5% fish gelatin
- c. Chicken anti-Neu5Gc diluted in 0.5% fish gelatin, different dilutions are used for human tissue (which has very little and needs the 3 step method) and for mouse tissue
- d. Chicken anti-Neu5Gc diluted in 0.5% fish gelatin containing 10% chimpanzee serum
- e. Extra slides as needed, for mild periodate, NaOH or sialidase treatment

**Methods:**

Air Dry – overnight/60 min. @ RT

If needed Prepare negative controls:

1. Sodium hydroxide (NaOH) to remove O-acetyl groups:
   - 1. treat one set with 0.1M NaOH @ room temperature (RT) - 30 min
   - followed by 3 buffer washes.
2. mild Sodium Periodate (NaI/O₄) treatment to truncate side chain of sialic acid:
   - make 2mM sodium periodate (107mg in 250ml of PBS) – 1 hour prior to use and let the solution sit in the dark at 4 °C
   - immerse sections on slides for 30 min, followed by 3 buffer washes

**Blocking:**

A. Block Endogenous Peroxidases of rbcs in vessels with H₂O₂ Fisher- H325-100 Opened:
   - Immerse slides in 0.03% H₂O₂ in PBST – 30 min at room temperature
   - followed by 3x Buffer Rinses in PBST

**Other negative controls:**

- a. Alter surface sialic acids by immersion in 2mM NaI/O₄
   - Immerse one set of sections on slides in 2mM periodate, in dark - 30 min.
   - followed by 3 buffer washes
- b. Remove surface sialic acids with Sialidase treatment of sections (see attached protocol)
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B. Collagen Block:
   a. Overlay each section with 0.5% Fish Gelatin in 1X PBST
   b. Tap off fish gelatin one slide at a time and
   c. Overlay sections with the following primary reagents

C. Block Endogenous Biotin: Vector- SP2001 Exp:
   a. 0.1% Avidin – 15 min. → buffer rinses – 3x
   b. 0.01% Biotin – 15 min. → buffer rinses – 3x

Fixation:
   d. 10% Neutral Buffered Formalin (NBF) -30 min. @ RT Fisher- SF93-4
   e. 3x Buffer Rinses PBST

Primary Reagents (separate sets):

<table>
<thead>
<tr>
<th>Primary Reagents</th>
<th>Dilutions</th>
<th>Incubation Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5% Fish gelatin/PBST</td>
<td>prediluted</td>
<td></td>
</tr>
<tr>
<td>Chicken IgY Jackson 003-000-003</td>
<td>1:500</td>
<td></td>
</tr>
<tr>
<td>Chicken Anti-Neu5Gc – prediluted aliquots @ 1:10 for use on HUMAN frozen sections @ 1:50 (1:500 final/actual dilution) OR for use MOUSE tissue frozen sections, it will be diluted @ 1:2,000 (1:20,000 final/actual dilution)</td>
<td>1:50 (human) OR 1:2,000 (mouse)</td>
<td>60 min. @ RT Always in covered humid chamber</td>
</tr>
<tr>
<td>Chicken Anti-Neu5Gc + 10% Goat serum inhibitor overlay with chicken anti-Neu5Gc, diluted in 0.5% fish gelatin containing 10% chimpanzee serum</td>
<td>1:50 (human) OR 1:2,000 (mouse)</td>
<td></td>
</tr>
<tr>
<td>f. 3x Buffer Rinses PBST</td>
<td></td>
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</tr>
</tbody>
</table>

9. Secondary Reagent:

<table>
<thead>
<tr>
<th>Biotinylated Donkey Anti-Chicken Jackson 703-065-155</th>
<th>1:500</th>
<th>30 min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>h. 3x Buffer Rinses PBST</td>
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Tertiary Reagent:

<table>
<thead>
<tr>
<th>HRP Streptavidin Jackson 016-030-084</th>
<th>1:500</th>
<th>30 min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>i. 3x Buffer Rinses PBST</td>
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</table>

Substrate:
If using HRP label the Chromagen is AEC Vector- SK 4200 Exp:
   Watch to see if negative control starts turning red color.
   If no color in negative control, for AEC, let slides go from 20 – 40 min.
   3x Buffer Rinses PBST

Nuclear Counterstain: Mayer’s Hematoxylin (is alcohol soluble) Sigma-Aldrich MHS32
   Incubation Time: 1 min.
   3x Buffer Rinses PBST

Mount in Aqueous Mounting Media (Vectamount) to view and acquire and record digital images
Examples of HRP IHC with anti-Neu5Gc, with or without mammalian serum block: