

Chicken anti-Neu5Gc on frozen

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:
2. treat one set with 0.1M NaOH @ room temperature (RT) - 30 min followed by 3 buffer washes.
3. mild Sodium Periodate (NaIO₄) 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 rbc's 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 NaIO₄**

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):

Dilutions:

Incubation Time:

0.5% Fish gelatin/PBST Sigma-Aldrich G7765-250ML	prediluted	60 min. @ RT Always in covered humid chamber
Chicken IgY Jackson 003-000-003	1:500	
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)	1:50 (human) OR 1:2,000 (mouse)	
Chicken Anti-Neu5Gc + 10% Goat serum inhibitor overlay with chicken anti-Neu5Gc, diluted in 0.5% fish gelatin containing 10% chimpanzee serum	1:50 (human) OR 1:2,000 (mouse)	

f. 3x Buffer Rinses PBST

g.

Secondary Reagent:

Biotinylated Donkey Anti-Chicken Jackson 703-065-155	1:500	30 min.
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h. 3x Buffer Rinses PBST

Tertiary Reagent:

HRP Streptavidin Jackson 016-030-084	1:500	30 min.
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i. 3x Buffer Rinses PBST

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

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Results, Future Plans, Notes:

Completed by:

Approx. Time:

Examples of HRP IHC with anti-Neu5Gc, with or without mammalian serum block:

