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Neuropathy Phentoyping Protocols - Apoptag (Peroxidase) Sections on Slides

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1 Works for me dx.doi.org/10.17504/protocols.io.3jkgkkw

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ABSTRACT

Summary:

Phenotyping of Rodents for the Presence of Diabetic Neuropathy
In man, the development of diabetic neuropathy is dependent on both the degree of glycemic control and the duration of diabetes. Diabetic neuropathy is a progressive disorder, with signs and symptoms that parallel the loss of nerve fibers over time. Consequently, assessments of neuropathy in mice are not performed at one time point, but are characterized at multiple time points during a 6 month period of diabetes. The degree of diabetes is evaluated in 2 ways: tail blood glucose measured following a 6 hour fast and glycated hemoglobin levels. The initial degree of neuropathy is screened using the methods discussed below. Detailed measures of neuropathy are employed when the initial screening instruments indicate a profound or unique phenotypic difference. This document contains protocols used by the DiaComp staff to examine and measure diabetic neuropathy at the whole animal, tissue and cellular levels.

Diabetic Complication:

EXTERNAL LINK

https://www.diacomp.org/shared/document.aspx?id=54&docType=Protocol

MATERIALS TEXT

Reagents:
- DNase
- DN buffer (30 mM Tris base, pH 7.2, 4 mM $MgCl_2$, 0.1 mM dithiothreitol)
- 4% paraformaldehyde
- Phosphate buffered saline (0.1 M pH 7.2, 150mM NaCl)
- 30% H_2O_2
- Proteinase K 3 ug/ml
- DAB or AEC
- Counterstain
- 50, 70, 95, 100% ETOH
- Hemo-De
- Cover glass
- Permount

Supplies:
- Slotted Wheaton dishes or Coplin jars

• Square Wheaton dishes or Coplin jars
• Slide racks and handles
• Humid chamber
• Oven
• Ice and ice bucket
• Pap pen
• Pipetmen, pipet tips, microfuge tubes etc

Kit (Intergen)
• Equilibration buffer aliquots, -20ºC
• TdT enzyme, aliquots, -20ºC
• Reaction buffer, aliquots, -20ºC
• Stopwash buffer, aliquots,-20ºC
• POD tagged anti-digoxygenin antibody 4ºC

Preparation:

1 1. Thaw equilibration buffer, TdT, reaction buffer and stopwash on ice.

2. Prepare working strength TdT enzyme.

3. Thaw enough reaction buffer and TdT to cover 30 ul/well TdT: reaction buffer, 33 µl: 77 µl.

4. Prepare stopwash buffer, add 1 ml buffer to 34 ml of ddH_20.

5. Prepare a humidified chamber.

6. Prepare anti-digoxygenin antibody.

7. Set oven to 37ºC.

8. Prepare DNase

Procedure:

2 1. Thaw and label sections, bench top or warm plate.

2. Ring sections with Pap Pen.

3. Specify 1 slide as positive and 1 slide as negative control.

This is a must for every set of TUNEL slides.

4. If sections or cells have not been fixed, fix with 4% paraformaldehyde for 10 min followed by 3 X 10 min rinses in PB.

5. If sections or cells already fixed, hydrate in PBS for 5 minutes.

6. Quench in 0.5% H_2O_2 for 5 minutes. 5 ml 30% H_2O_2 in 300 ml PBS. If using a fluorescence kit, skip this step.

7. Rinse twice for 5 minutes in PBS.

8. Place Positive and Negative control slides in DN buffer for 5 min.

9. Apply DNase (1.0/0.1 ug/ml DN buffer) to POSITIVE and NEGATIVE CONTROL SLIDES ONLY for 10 minutes at 22ºC.

10. Treat the rest of slides with Proteinase K, 3 ug/ml 10 min.

11. Rinse all slides in PBS for 3 X 5 min keep DNase treated slides separate until the end of this set of rinses.

12. Apply 30 µl of equilibration buffer to each well.

13. Incubate at 22°C for 15 minutes.

14. Blot off excess equilibration buffer and add 30 µL/well TdT in reaction buffer. **Negative control does not get TDT solution, incubate with PBS.**

15. Incubate for 1 in humid chamber @ 37°C.

16. Place slides in stop wash buffer and incubate for 10 min at 22ºC.

17. Rinse 2 X 5 min in PBS.

18. Remove from PBS and gently touch corner of well with filter paper wedge to remove excess fluid.

19. Add Anti-Dig antibody (30 µL) for 1 hour at 22ºC.

20. (For peroxidase method, take DAB and glucose oxidase out of freezer at this point, follow DAB Protocol. For fluorescence method, prepare Prolong, see Prolong Protocol).

21. Wash in PBS 3 X 10 min.

Notes: Never let slides dry out ever!!!

Figure 1

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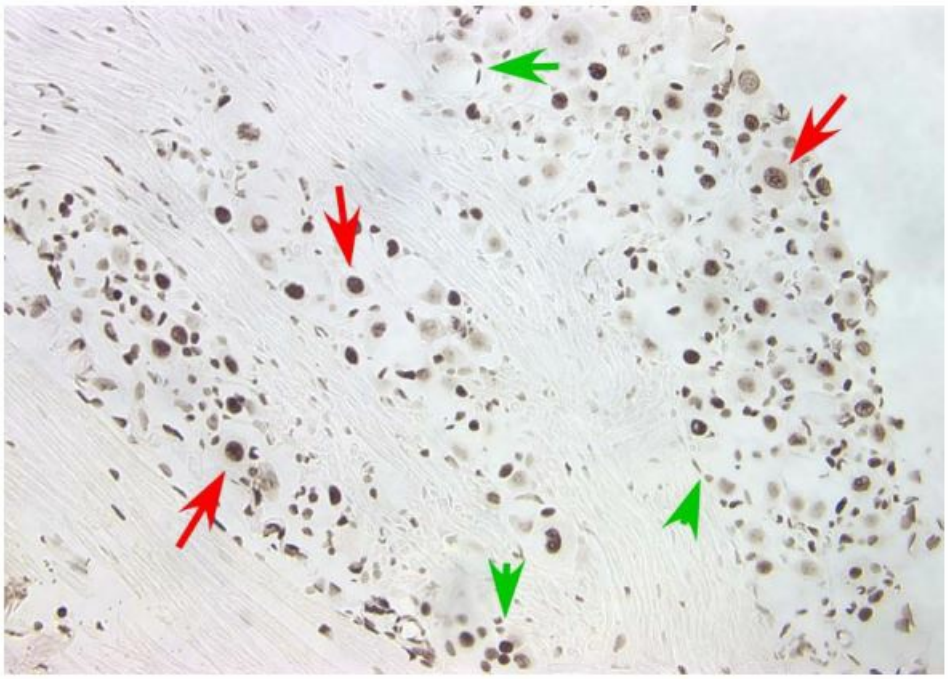

DNAse treatement followed by TdT endlabeling serves as a positive control for the TUNEL reaction. All nuclei are labeled including neurons (red arrows) and satellite cells (green arrows). Experimental sections will demonstrate a range of reactivity and number of positive cells.