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Neuropathy Phentoyping Protocols - Immunofluorescence Method for 8DG Localizaiton

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

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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:

Reference:
Yarborough et al, Cancer Res. 56:683-688, 1996

EXTERNAL LINK

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

MATERIALS TEXT

Reagents:
• RNase
• Proteinase K
• HCl
• Ethos, 50, 70, 95, 100%
• Hemo-De
• PBS
• Tris (10 mM, pH 7.5, 1 mM EDTA, 0.4 M NaCl)

Counterstains:
• Bis-benzimide

Other Chemicals:

• Normal serum
• Non-fat dry milk
• Bleach
• H_2O_2

Mounting Media:
• Prolong
• Gelmount

Glassware:
• Square Wheaton dishes
• Slide racks and handles
• Coplin jars
• Beakers
• Microfuge tubes

1 Deparaffinize slides by soaking in Hemo-D overnight in rocker oven. If using cryosections, thaw on warm plate 10 min, ring with PAP pen.

2 Re-hydrate through EtOH 100% -50% dH_2O.

3 Equilibrate sections in Tris. (10 mM, pH 7.5, 1 mM EDTA, 0.4 M NaCl)

4 Incubate with RNase in Tris, 100 µg/ml, 37ºC, 1 hour.

5 Rinse 2 X 10 min Tris.

6 Incubate with Proteinase K 10 µg/ml Tris 22ºC, 7 min.

7 Rinse 2 X 10 min Tris.

8 Incubate in 4 N HCl 22ºC, 7 min.

9 Rinse 2 X 10 min Tris.

10 Rinse 2 X 10 min PBS.

11 Begin normal IHC procedure with the 8DG antibody.