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BDA Histology

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Blake Butler¹

¹University of Western Ontario



Blake Butler

University of Western Ontario

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Protocol status: Working

We use this protocol and it's working



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Keywords: bda histology, feline tissue preparation, staining protocol, tissue, cryoprotected, perfusion

Abstract

Staining protocol for visualization of BDA 3K.

Species - Feline

Tissue Preparation - Perfusion of 4% PAF; Cryoprotected 30% Sucrose; Section thickness of 60 microns

Materials

STEP MATERIALS

⊗ 3,3'-Diaminobenzidine tetrahydrochloride **Merck MilliporeSigma (Sigma-Aldrich) Catalog #D5905**

⊗ Avidin/Biotin HRP Complex **Vector Laboratories Catalog #PK-6100**

⊗ Normal Goat Serum **Cedarlane Catalog #CL1200-500**

Protocol materials

⊗ 3,3'-Diaminobenzidine tetrahydrochloride **Merck MilliporeSigma (Sigma-Aldrich) Catalog #D5905**

⊗ Avidin/Biotin HRP Complex **Vector Laboratories Catalog #PK-6100**

⊗ Normal Goat Serum **Cedarlane Catalog #CL1200-500**



Preparatory Steps

1 Reconstitute the Vectastain ABC solution

Prepare 80 mL solution:

 79 mL  0.1 Molarity (M) Physiological Buffer

 480 μ L Solution A

 480 μ L Solution B

 Avidin/Biotin HRP Complex **Vector Laboratories Catalog #PK-6100**

Thaw 6 $\times$ 1mL vials of NGS for later use

 Normal Goat Serum **Cedarlane Catalog #CL1200-500**

Histological Reaction

2

2.1 Rinse sections in 0.1 M Physiological Buffer (4 $\times$ 5min)

 00:05:00

 00:05:00

 00:05:00

 00:05:00

2.2 Block Endogenous Peroxidase

 105 mL Methanol

 45 mL De-ionized Water

 750 μ L  30 % volume Hydrogen Peroxide

 00:30:00



2.3 Rinse sections in 0.1 M Physiological Buffer (4×5min)

00:05:00

00:05:00

00:05:00

00:05:00

2.4 Transfer tissue to individual reaction wells and incubate in Avidin/Biotin HRP Complex Prepared in Step 1

01:30:00

2.5 Transfer tissue back to reaction trays and rinse sections in 0.1 M Physiological Buffer (3×10min)

00:10:00

00:10:00

00:10:00

2.6 Incubate in DAB-Nickel Chromogen Solution

150 mL [M] 0.1 Molarity (M) Physiological Buffer

.1125 g 3,3' Diaminobenzidine Tetrachloride (DAB)

1.875 mL [M] 1 Mass / % volume Cobalt Chloride (Added dropwise)

3 mL [M] 1 Mass / % volume Nickel Ammonium Sulfate (Added dropwise)

50 µL [M] 30 % volume Hydrogen Peroxide (Added 60s before incubation)

 3,3'-Diaminobenzidine tetrahydrochloride **Merck MilliporeSigma (Sigma-Aldrich) Catalog #D5905**

00:20:00 (Approximate time - Actual endpoint determined visually)

2.7 Rinse sections in 0.1 M Physiological Buffer (3×5min)



⌚ 00:05:00

⌚ 00:05:00

⌚ 00:05:00

3 Mount from 0.01 M Physiological Buffer