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BDA Histology

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1 Works for me dx.doi.org/10.17504/protocols.io.z4af8se

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ABSTRACT

Staining protocol for visualization of BDA 3K.
Species - Feline
Tissue Preparation - Perfusion of 4% PAF; Cryoprotected 30% Sucrose; Section thickness of 60 microns

STEPS MATERIALS

NAME	CATALOG #	VENDOR
Avidin/Biotin HRP Complex	PK-6100	Vector Laboratories
Normal Goat Serum	CL1200-500	Cedarlane
3,3'-Diaminobenzidine tetrahydrochloride	D5905	Sigma Aldrich

Preparatory Steps

1 Reconstitute the Vectastain ABC solution

Prepare 80 mL solution:

79 ml [M]**0.1 Molarity (M)** Physiological Buffer

480 µl Solution A

480 µl Solution B

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

Thaw 6 x 1mL vials of NGS for later use

Normal Goat Serum
by Cedarlane
Catalog #: CL1200-500

Histological Reaction

2

2.1 Rinse sections in 0.1 M Physiological Buffer (4x5min)

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 µl [M]30 % volume Hydrogen Peroxide

00:30:00

2.3 Rinse sections in 0.1 M Physiological Buffer (4x5min)

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 (3x10min)

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

0.1125 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
by Sigma Aldrich
Catalog #: D5905

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

2.7 Rinse sections in 0.1 M Physiological Buffer (3x5min)

00:05:00

00:05:00

00:05:00

3 Mount from 0.01 M Physiological Buffer