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Preparation and Immunohistochemistry of Mouse Small Intestine and Colon

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We use this protocol and it's working

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Abstract

Mice were euthanized, the small and large intestine collected, and fixed frozen sections prepared. The microscope mounted tissue sections were stained using immunohistochemistry. Expression of specific proteins and reporter proteins were visualized using microscopy.

Materials

Primary Antibodies

	A	B	C	D	E	F
	Target	Host	Dilution	Manufacturer	Catalog number	RRID
	Serotonin	Rabbit	1:5000	ImmunoStar	Cat# 20080	RRID:AB_572263
	Serotonin	Goat	1:500	Abcam	Cat# ab66047	RRID:AB_1142794
	GFP	Chicken	1:500	Aves Labs	Cat# GFP-1010	RRID:AB_2307313
	dsRed	Rabbit	1:500	Takara Bio	Cat# 632496	RRID:AB_10013483

Secondary Antibodies

	A	B	C	D	E	F
	Conjugate/Target	Host	Dilution	Manufacturer	Catalog number	RRID
	AF647 anti-rabbit	Donkey	1:500	Invitrogen	Catalog # A32795	RRID:AB_2536183
	AF647 anti-goat	Donkey	1:500	Invitrogen	Catalog # A-21447	RRID:AB_141844
	AF488 anti-chicken	Donkey	1:500	Invitrogen	Catalog # A78948	RRID:AB_2921070
	AF564 anti-rabbit	Donkey	1:500	Invitrogen	Catalog # A10042	RRID:AB_2534017

Other Reagents

⊗ OCT (Optimal Cutting Temperature compound) **Sakura Finetek Catalog #4583**

⊗ Donkey Serum **Merck Millipore (EMD Millipore) Catalog #S30-100ML**

⊗ PBS - Phosphate-Buffered Saline (10X) pH 7.4 **Thermo Fisher Scientific Catalog #AM9625**

⊗ Triton X-100 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #T8787-50ML**

⊗ Bovine Serum Albumin **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A7030**

⊗ Sucrose **Merck MilliporeSigma (Sigma-Aldrich) Catalog #S7903**



 Prolong Gold **Thermo Fisher Scientific Catalog #P36930**

 Paraformaldehyde 32% (methanol free) **Electron Microscopy Sciences Catalog #15714**

Composition of Abbreviated Solutions

PBS: phosphate-buffered saline, 0.1 M, pH 7.2

Blocking buffer: 5% donkey serum, 5% BSA, .3% Triton X-100 in PBS

Tissue Collection and Preparation

- 1 Mice 6 - 8 weeks old were euthanized by isoflurane administration followed by cervical dislocation.
- 2 Mice were transcardially perfused with 5ml ice cold PBS.
- 3 Whole small intestine and colon were collected and kept in PBS on ice. The small intestine was dissected into three segments of approximately equal length.
- 4 Each segment (proximal, medial, and distal small intestine and whole colon) was flushed with 5ml ice cold PBS, cut lengthwise, and laid flat with the mucosal surface facing up. Each segment was further washed with ice cold PBS.
- 5 Tissue segments were washed with ice cold 4% PFA in PBS, and then laid flat in between two layers of filter paper and fixed in 4% PFA in PBS at 4C for either 4hrs or 24hrs.
- 6 Tissue was rinsed in excess PBS and transferred to 30% sucrose solution for at least 16 hours.
- 7 Tissue was transferred to a mixture of 50% OCT, 15% sucrose in PBS and rolled on a wooden stick.
- 8 Tissue was frozen in OCT as a "swiss roll" and sectioned into 5um or 20um sections on a cryostat and kept at -80C.

Immunohistochemistry

- 9 Slide-mounted tissue sections were thawed and air dried for 30 minutes
- 10 Sections were washed twice for 10 minutes in PBS and once for 10 minutes in .2% Triton X-100 in PBS.
- 11 Sections were blocked for 1 hour in blocking buffer at room temperature.
- 12 Sections were incubated with primary antibodies in blocking buffer overnight at 4C.



- 13 Sections were washed thrice for 10 minutes in .2% Triton X-100 in PBS.
- 14 Sections were incubated with secondary antibodies and DAPI in blocking buffer for 1-2 hours at room temperature.
- 15 Sections were washed twice for 10 minutes in .2% Triton X-100 in PBS and twice for 10 minutes in PBS.
- 16 Sections were mounted in Prolong Gold Mounting Media.