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Immunohistochemistry

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ABSTRACT

Outlines procedures to perform immunohistochemistry on tissue that had been treated with AAV.

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Protocol status: Working We use this protocol and it's working

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PROTOCOL integer ID:

81725

Tissue slicing via vibratome (mice and rhesus macaque)

All tissue should be stored in PBS prior to slicing and further processing. Remove tissue and

1

prepare for mounting. For mouse brain, we separate each half hemisphere. Rhesus macaque brain was separated into 4 mm thick coronal blocks. For all other tissue (e.g. liver), remove a 4-5 mm piece of tissue and cut one edge to make a flat surface.

2 Use super glue to mount the tissue on the provided mounting disk and fill reservoir with 1X PBS.

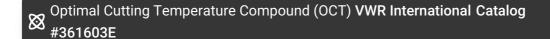


3 Slice tissue to 50-100 um slice and collect in 24- (mouse) or 6- (rhesus macaque) well plates filled with 1X PBS.

Tissue can also be cut to 300 um to be used for index refraction matching (e.g. to perform morphological reconstructions).

Tissue slicing via cryostat (marmoset and rhesus macaque)

- All tissue should be stored in PBS prior to slicing and further processing. Remove tissue and incubate in 30% sucrose at 4 °C on a shaker. For marmoset tissue, incubate overnight. For rhesus macaque tissue, incubate for 72 hours, changing the 30% sucrose solution after 48 hours.
- 5 Embed tissue in OCT using 100% isopropanol (or similar) chilled with dry ice. Orient tissue to ensure coronal slices.



Equipment	
Peel Away Disposable Embedding Molds	NAME
Embedding mold	TYPE
Electron Microscopy Sciences	BRAND
70182	SKU
https://www.emsdiasum.com/peel-away-disposable-embedding-molds	LINK

Equipment	
Peel Away Disposable Embedding Molds	NAME
Embedding mold	TYPE
Electron Microscopy Sciences	BRAND
70184	SKU
https://www.emsdiasum.com/peel-away-disposable-embedding-molds	LINK

Equipment	
Super Mega Cassette Stainless Steel Base Molds	NAME
Embedding mold	TYPE
Ted Pella Inc.	BRAND
27197-2	SKU
https://www.tedpella.com/Embedding_html/Peel-A- Way_Disposable_Histology_Molds.aspx#anchor27197	LINK

Equipment	
Super Mega Cassette Stainless Steel Base Molds	NAME
Embedding mold	TYPE
Ted Pella Inc.	BRAND
27197-3	SKU
https://www.tedpella.com/Embedding_html/Peel-A- Way_Disposable_Histology_Molds.aspx#anchor27197	LINK

- 6 Store tissue at ****** -80 °C overnight before continuing to slicing.
- 7 Cut tissue to 50 um slice and collect in 24- (marmoset) or 6- (rhesus macaque) well plates filled with 1X PBS.

Immunohistochemistry

8 Incubate slices in primary antibody overnight on a shaker at room temperature. Dilute antibody in 1X PBS supplemented with 0.1% Triton X-100

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

and 10%

normal donkey serum

Normal Donkey Serum **Jackson ImmunoResearch Laboratories**, **Inc. Catalog #017-000-** 121

A	В	С	D	E
Antibody epitope	Vendor	Cat #	RRID	Dilution
GLUT1	Millipore- Sigma	07-1401	AB_1587074	1:200

A	В	С	D	E
НА	Cell Signaling Technology	3724	AB_1549585	1:200
GFP	Aves Labs	GFP-1020	AB_10000240	1:500
NeuN	Abcam	ab177487	AB_2532109	1:200
S100□	Abcam	ab52642	AB_882426	1:200

- **9** Wash with PBS 3-5 times at room temperature on a shaker.
- Incubate slices in secondary antibody overnight on a shaker at room temperature. Dilute antibody in 1X PBS supplemented with 0.1% Triton X-100 and 10% normal donkey serum.

Protect from light to avoid photobleaching.

A	В	С	D	E
Antibody epitope	Vendor	Cat #	RRID	Dilution
rabbit IgG	Jackson Immunoresea rch Labs	711-605-152	AB_2492288	1:200
chicken IgY	Jackson Immunoresea rch Labs	703-605-155	AB_2340379	1:200

11 Wash with PBS 3-5 times at room temperature on a shaker.

Protect from light to avoid photobleaching.

Mount sections on slides and allow slice to dry at room temperature in the dark. Mount coverslip using Prolong Diamond Antifade.

ProLong Diamond Antifade Mountant Thermo Fisher Scientific Catalog #P36970