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## Immunohistochemistry

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**Protocol status:** Working

We use this protocol and it's working

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Aligning Science Across Parkinson's

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## Abstract

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

## Protocol materials

✂ Optimal Cutting Temperature Compound (OCT) **VWR International (Avantor) Catalog #361603E**

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

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

✂ ProLong Diamond Antifade Mountant **Thermo Fisher Scientific Catalog #P36970**

## Troubleshooting

## Tissue slicing via vibratome (mice and rhesus macaque)

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

### Equipment

|  |       |
|--|-------|
| Leica VT1200 S Fully automated vibrating blade microtome | NAME  |
| Vibratome  | TYPE  |
| Leica  | BRAND |
| VT1200S  | SKU   |


<https://www.leicabiosystems.com/us/research/vibratomes/leica-vt1200-s/><sup>LINK</sup>

- 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)

- 4 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.



Optimal Cutting Temperature Compound (OCT) **VWR International**  
**(Avantor) Catalog #361603E**

| Equipment   |       |
|---|-------|
| Peel Away Disposable Embedding Molds  | NAME  |
| Embedding mold  | TYPE  |
| Electron Microscopy Sciences  | BRAND |
| 70182   | SKU   |
| <a href="https://www.emsdiasum.com/peel-away-disposable-embedding-molds">https://www.emsdiasum.com/peel-away-disposable-embedding-molds</a> <small>LINK</small> |       |

| Equipment   |       |
|---|-------|
| Peel Away Disposable Embedding Molds  | NAME  |
| Embedding mold  | TYPE  |
| Electron Microscopy Sciences  | BRAND |
| 70184   | SKU   |
| <a href="https://www.emsdiasum.com/peel-away-disposable-embedding-molds">https://www.emsdiasum.com/peel-away-disposable-embedding-molds</a> <small>LINK</small> |       |



## 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](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](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                | B                         | C        | D           | E        |
|--|------------------|---------------------------|----------|-------------|----------|
|  | Antibody epitope | Vendor                    | Cat #    | RRID        | Dilution |
|  | GLUT1            | Millipore-Sigma           | 07-1401  | AB_1587074  | 1:200    |
|  | HA               | 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 $\beta$     | Abcam                     | ab52642  | AB_882426   | 1:200    |

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

- 10 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                | B                           | C           | D          | E        |
|--|------------------|-----------------------------|-------------|------------|----------|
|  | Antibody epitope | Vendor                      | Cat #       | RRID       | Dilution |
|  | rabbit IgG       | Jackson ImmunoResearch Labs | 711-605-152 | AB_2492288 | 1:200    |




|  | A           | B                                 | C               | D              | E     |
|--|-------------|-----------------------------------|-----------------|----------------|-------|
|  | chicken IgY | Jackson<br>ImmunoResearch<br>Labs | 703-605-<br>155 | AB_234037<br>9 | 1:200 |

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

Protect from light to avoid photobleaching.

- 12 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**