



May 14, 2024

## Enzymatic treatment of free floating sections

DOI

[dx.doi.org/10.17504/protocols.io.81wgbzyyqgpk/v1](https://dx.doi.org/10.17504/protocols.io.81wgbzyyqgpk/v1)

Bryan Killinger<sup>1</sup>

<sup>1</sup>Rush University



Bryan Killinger

Rush University

### Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.81wgbzyyqgpk/v1>

**Protocol Citation:** Bryan Killinger 2024. Enzymatic treatment of free floating sections. **protocols.io**  
<https://dx.doi.org/10.17504/protocols.io.81wgbzyyqgpk/v1>

**License:** This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

**Protocol status:** Working

We use this protocol and it's working

**Created:** May 14, 2024



**Last Modified:** May 14, 2024

**Protocol Integer ID:** 99781

**Keywords:** floating brain section, floating sections general protocol, brain section, sections general protocol for enzymatic treatment, enzymatic treatment

**Funders Acknowledgements:**

**Michael J. Fox Foundation**

Grant ID: ASAP-021030

**NINDS**

Grant ID: 1R01NS128467

## Abstract

General protocol for enzymatic treatment of free floating brain sections.

## Troubleshooting

**Prepare 40 micron thick free floating brain sections for enzymatic treatments.****30m**

1 Wash in DM

10m

2 Wash in DM

10m

3 Wash in DM

10m

**Equilibrate tissue sections in reaction buffer.****10m**

4 For GluC treatment, incubate in 1X GluC reaction buffer (New England Biolabs)

5m

5 For Trypsin treatment, incubate in TBS (50mM Tris-HCl, pH 7.6, 150mM NaCl)

5m

**Enzymatic Treatment****1d 8h**

6 For Gluc, combine 100ul 2X GluC reaction buffer and 100ul GluC (New England BioLabs, 100 ng/ul dissolved in high-purity water). Incubate tissues with mixture at 37C for 16h.

16h

7 For Trypsin, combine 4ul of trypsin stock (Sigma-Aldrich, 1 mg/ml dissolved in 1 mM HCl, pH 3) and 196ul of TBS. Incubate tissues with the mixture at 37C for 16h.

16h

**Wash****30m**

8 wash tissue in DM

10m

9 wash tissue in DM

10m

10 wash tissue in DM

10m