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# Enzymatic treatment of free floating sections

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

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## Abstract

General protocol for enzymatic treatment of free floating brain sections.

## 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/µl 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