

TUNEL Protocol using TACS TdT reagents

1 Fix 3.7% Formaldehyde in 1X PBS 30 min RT

- Add FRESH fix for 4 more hours
- 1X PBS wash 20 min
- 1X PBS wash 20 min

2 Infiltrate for OCT embedding

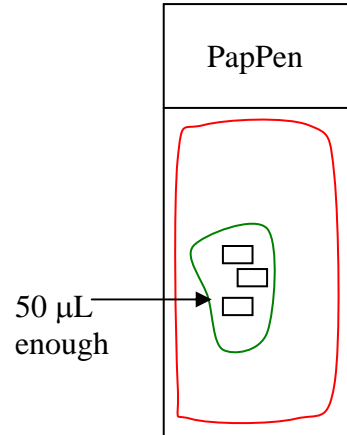
- 5% sucrose/1X PBS 30 min
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- 5% sucrose/1X PBS 30 min
- 30% sucrose/1X PBS 30 min or O/N 4°C
- 30% sucrose/1X PBS:OCT 1:1 4 hrs or O/N 4°C

3 Embed in OCT

- 100% OCT and freeze

4 Section

- 12-14 μm 2 PER SLIDE
- Dry on warmer (50°C) 30 min – 2 hrs
- Outline with PapPen



5 Permeablize tissue (Neuropore shelf of 4°C)

- Wash in 1X PBS in coppin jar
- Add 50 μL Neuropore reagent on each section in humidified chamber 75 min RT
- Wash 1X PBS 2 min
- Wash 1X PBS 2 min

6 Label with TdT

- Incubate in 1X TdT Label Buffer 5 min
- Add 50 μL label mix 1 hr at 37°C (humidified)

LABEL MIX/1 SECTION

1 μL TdT-dNTP	-20°C III
1 μL Ion	-20°C III
1 μL TdT enzyme	-20°C II
50 μL 1X label buffer	4°C

7 Stop Reaction

- Incubate in coppin jar full of 1X stop buffer 5 min
- Wash 2 min 1X PBS
- Wash 2 min 1X PBS

8 StrepAvidin-(FITC) label (4°C fridge)

- 50 μL StrepA-F on each section IN THE DARK 20 min
- Wash in coppin jar full of 1X PBS, 0.1% Tween-20 2 min
- Wash again 2 m in

StrepAvidin-FITC mix

2 μL StrepA-F
400 μL 1X PBS
0.5 μL TO-PRO-3