TUNEL labeling on mouse brain cryosections
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Notes: I use 20 um thick sections; for quantification, I label a series of 10 sections per brain, count all positive cells, and calculate the average # of TUNEL+ cells per section per brain.

1. Air dry slides on benchtop 5-10 min.
2. Rinse in PBS 10 min.
3. Immerse slides in 10 mM Citrate buffer pH 6.0 in a plastic microwaveable dish, and bring to a boil 3 times (add a little fresh buffer on top after the first 2 boils).
4. Cool slides to room temp in Citrate buffer for 15 min.
5. Quickly dip slides 2-3 times in PBS
6. Block for 30 min at room temp in humidified chamber in: 10% normal goat serum
3% BSA
in 0.1M Tris pH 7.5
7. Dip slides quickly in PBS
8. Remove as much PBS from slides as possible, and place horizontally in humidified chamber. Apply 120 uL TUNEL reaction mixture (see below) to each slide, and coverslip. Wrap humidified chamber with foil, and incubate at 37C for 1 hr.
9. Remove coverslips in PBS or by allowing the slips to fall off slide. Rinse slides 3 x 5 min in PBS
10. Mount slides with Vectashield and coverslip.

To make 10 mM Citrate buffer:
1) Add 1.92 g anhydrous citric acid ($C_6H_8O_7$) to 900 mL dH_2O.
2) pH to 6.0 with approximately 2.6 mL 10N NaOH.

To order TUNEL labeling mix:
Roche In situ cell death detection kit, fluorescein (Cat no. 11684795910) price= $372.00
or Roche In situ cell death detection kit, TMR red (Cat no. 12156792910)

To make the TUNEL reaction mix:
1) Make this up 10 minutes or less before applying to slides!
2) Add 19 parts labeling mix buffer to 1 part TdT enzyme (eg. per 120 uL, use 6 uL enzyme + 114 uL labeling mix buffer); mix thoroughly.