Sive Lab Protocol
March 2007

TUNEL Protocol

We use the Apoptag kit (ApopTag® PEROXIDASE IN SITU APOPTOSIS DETECTION KIT from Chemicon Cat# S7100). We don't use the peroxidase portion of the kit and instead use an alkaline phosphatase or FITC conjugated to DIG.

Day 1

- Fix in 4% PFA (paraformaldehyde) 45 min-1 hr
- Wash 3X PBS
- Dehydrate in ETOH series (10 min each) and store at -20C for 1 day min and up to 1 week max.

Day 2

- Re-hydrate in ETOH series (10 min each)
- Wash in PBS, 3X 10 min
- Dissect heads of embryos (or region you are focusing on)-this opens up embryo and allows better penetration of reagents.
- Bleach in 2X SSC + 1% H_2O_2 + 1% formamide (put in clear dishes on a light box until embryos are very white, if there are a lot of bubbles reduce amt of H_2O_2)
- Wash in PBS, 3X 10 min
- Treat with proteinase K, 1-2 ug/ml for 5-10 mins (this should be adjusted depending on stage)
- Wash in 2X PBS, 10 min
- Re-fix in 4% PFA, 15-20 min
- Wash 5X PBS 10 min each put into 1.5 ml tubes
- Remove as much PBS as possible and then add Equilibration Buffer provided in kit.
- Remove as much as possible then add Reaction Buffer +TdT enzyme (77 ul buffer and 33 ul enzyme)
- Incubate overnight at room temp.

Day 3

- Incubate for 1 hr at 37C
- Remove reaction solution and add 1ml of stop solution, incubate for 1hr.
- Wash in MAB 3X 10min
- Block in blocking solution; 8 volumes MAB: 1 volume BMB blocking solution (from Boehringer #1096176): 1 volume lamb serum for 2 hrs
- Remove blocking solution and add antibody. DIG-AP (1:2000) or DIG-FITC (1:10) (both from Roche) and incubate for 2-3 days at 4C

Day 6

- Wash in MAB 5X 30 min (it is even better if washed overnight at 4C)
- If this is fluorescent (DIG-FITC) then can view immediately
- Incubate in AP buffer for 1 hr then do NBT/BCIP staining as you would for in situ hybridization (36ul NBT and 28ul BCIP in 10ml AP buffer).

Notes:

- Washes work best if done with large volumes using Corning® Netwells® inserts (see Sigma CLS3480).
- Put embryos in 1.5 ml tubes for enzyme and antibody labeling to save reagents.
- Using methanol rather than ethanol for storing embryos may prevent problems with bubbles in the bleaching step but you may get reduced apoptosis labeling.
- Add tween-20 to your PBS (0.1-1%) if your embryos are sticking to pipettes etc.
- AP buffer, 2X SSC, MAB, AP and PBS are the same buffers as used for in situ hybridization and/or immunohistochemistry and can be found in most protocols for fish and frogs (see: Early development of *Xenopus laevis*: a laboratory manual. Sive HL, Grainger R, Harlard R. Cold Spring Harbor Laboratory Press, 2000.).