# Fisher Lab Apoptosis Fluorescent Protocol

## ***Solutions***

1. Make working strength TDT enzyme. Mix **77ul** of *reaction buffer* with **33ul** of *TDT enzyme* in **390ul** of PBS. Mix well by vortexing.
2. Make Stop-Wash buffer. (**1ml** of *Stop Wash buffer* into **34ml** of *distilled water*.)

## ***Procedure (Keep wrapped in foil throughout the procedure)***

1. Thoroughly wash specimen in PBS (minimum of 2 hrs. with several washes in between.)
2. Immediately apply 75**ul** *equilibration buffer* in addition to 425 **ul** of PBS for a total of 500 **ul** of solution directly onto the specimen. Incubate for 3 minutes at room temperature.
3. Remove equilibration buffer, apply working strength *TDT enzyme (solution #1),* wrap in aluminum foil and label (Fisher Lab x 3611, Name, Date) and place in incubator at 37 degree (located on 5$^{th}$ floor) for 1 hour.
4. Remove TDT enzyme, and apply *stop-wash buffer (solution #2)*. Gently shake for 30 seconds, followed by 10 min. incubation at room temperature.
5. Wash the specimen 3 times for 2 minutes in PBS.

   While rinsing, make working strength Fluorscein Antibody Solution:

   Add **68ul** of *blocking solution* to **62 ul** of *anti-Digoxigenin conjugate* for a total of **130ul**. Add **370ul** of PBS to bring total volume up to **500ul**. Keep refrigerated and in the dark for *no more than 1 hour*.

6. Add Fluroescein antibody and incubate in humidifying chamber (5$^{th}$ floor) for 40 minutes. (Avoid exposure to light , wrap in foil and label as explained above).
7. Wash specimen 4 times in PBS, 2 minuets per wash.
8. Mount.