

TUNEL technology --- Whole mount of mosquito midgut

Kit: In situ cell death detection kit, fluorescein (Roche Cat. No. 11 684 795 910)

1. Staging and dissection of midgut. Fix tissues in 4% paraformaldehyde in PBS (freshly prepared) at 4 °C O/N, or RT 1-2 hr.
2. Wash with 1X PBS three times, 5min each time. For storage, place tissues in 100% methanol and store at -20 °C.
3. Permeabilize tissues with 30ug/ml proteinase K(2ul 600mAU/ml or 40 mAU/mg protein in 1ml 10mM Tris-HCl pH8.0 buffer) for 5-10min at 37°C. [Or use 0.3% Triton X-100, 0.1% sodium citrate solution for 10 min, not quit well for midgut permeabilize].
4. Mix 2uL Enzyme solution and 18uL Label solution. Incubate tissues with this mixture for 60 min at 37 °C, shaking the tissue will be better.
5. Wash tissues with 1X PBS twice and mount them using 50% glycerol in PBS with 1000X DAPI.
6. Tissues were visualized under a confocal microscope.