

DAPI Protocol

Prepare Stock Solution:

- Add 1000 µl of molecular biology grade water to Dapi powder (10 mg/mL)
- Vortex for 30 seconds
- Cover tube with aluminum foil (light sensitive) and store at – 20°C

Prepare Intermediate Solution:

- Pipette 10 µl of original Dapi stock solution and add to a new microcentrifuge tube
- Add 990 µl of 1X PBS to make 1 ml of a 100 µg/ml solution
- Store at – 20°C

Prepare Working Solution:

- Add 5 µl of intermediate solution to 1.5 ml microcentrifuge tubes
- Add 995 µl of 1X PBS to make 1 ml of a 0.5 ug/ml solution
- Store at 4° C.

Stain embryos with Dapi

1. Add working solution (1 ml) to embryos in 1.5ml microcentrifuge tubes
2. Incubate embryos for 30 min at room temperature in the dark
3. Wash embryos twice with 1X PBS
4. Add three drops of mounting medium
5. Gently transfer embryos to slides, cover slides, and seal slides with nail polish
6. Cover slides with aluminum foil and view slides using the confocal microscope immediately after preparation
7. After use, transfer embryos to PBS and store at 4° C.

Reagents:

- Dapi (Biotium brand purchased from VWR, Cat# 89139-054)
- Mounting media (Shandon Aqua-mount Slide mounting media purchased from Fisher Scientific, Cat# 14-390-5)