

**PROTOCOL FOR DAPI STAINING**  
**(Corning 384 Square Well Black Microplate)**

1. Fix cells with 60 $\mu$ L/well of 4% PFA (Para Formaldehyde) for 8 minutes.  
**Note:** This timing is very critical and it should definitely not be for more than 10 minutes. This step should be done only in the Biosafety level 2 fume hood (hood on the right) since it involves volatile PFA. After this step, the PFA should be dumped onto paper towels that should be wrapped in aluminum foil before disposal.
2. Wash cells with 60 $\mu$ L/well of 1x PBS (Phosphate Buffered Saline), 3 times, 5 minutes / wash
3. Permeabilize cells with 60 $\mu$ L/well of 0.1% Triton-X-100 for 10 minutes
4. Repeat the 3 washes in Step #2
5. Stain cells with 50 $\mu$ L/well of DAPI (1:2000 dilution, in 1x TBST) for 5 minutes
6. Add 50 $\mu$ L/well of 1x PBS to keep the cells hydrated while imaging on the Image Express Micro

**Important Pointers**

- Between each step, aspirate cells by dumping into biohazard bin directly and proceeding onto the next step (except step 1, details given in step 1)
- For each step, fixing, washing, permeabilizing, staining, etc, the Corning 384 square well black microplate should be rotated using the Nutator.