IMMUNOLOGICAL SCIENCES Product Data Sheet

IS-7712 DAPI Stain Solution

Size 1 mL

Concentration: 10 mg/mL

Storage: Store DAPI Solution at 4°C, protected from light. Product is stable for at least one year from date of receipt when stored as recommended.

Molecular Info: Absorption/Emission: 358/461 nm (with DNA)

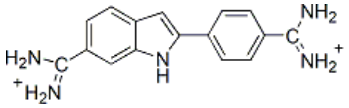

Figure 1. DAPI (4′,6-Diamidino-2-Phenylindole).

Description: DAPI is a blue DNA dye that is widely used as a nuclear counterstain for fluorescence microscopy, chromosome staining, and flow cytometry. The dye binds to the minor groove of dsDNA with approximately 20-fold fluorescence enhancement, with higher affinity for A-T rich regions. Because of the potential toxicity of DAPI, we offer DAPI as a ready-to-use solution in water as a safer alternative to weighing out the solid form. We also offer DAPI dilactate solid, which is more water soluble than the dihydrochloride salt of the dye.
At lower concentrations (~1 ug/mL), DAPI is impermeant to live cells, but useful as a nuclear counterstain in fixed cells or tissue sections. At higher concentrations (~10 ug/mL), DAPI can be used to stain live cells.

Staining Protocols

Live cell staining

Below we provide two protocols for staining live cells with DAPI. Staining by medium exchange results in uniform exposure of cells to probe. However, for some cell types, morphology or viability may be affected by medium exchange. In addition, floating dead cells may be lost during medium removal, and suspension cells must be collected by centrifugation to exchange the medium. Direct addition of 10X probe is a convenient staining method that doesn't require medium exchange, but care must be taken to mix immediately yet gently to avoid high transient probe concentration or disruption of cells by pipetting. Note that we do not recommend adding highly concentrated dye directly to cells in culture, as this will result in local areas of high dye exposure.

Live cell staining by medium exchange

1. Dilute DAPI to 10 ug/mL in fresh, complete culture medium. DAPI can be combined with other fluorescent probes.
2. Remove medium from the cells and replace with medium containing dye.
3. Incubate cells at room temperature or 37°C for 5-15 minutes, then image. Note: Washing is not necessary for specific staining, but nuclear staining is stable after washing.

For Research use only

Live cell staining by direct addition of 10X probe

1. Prepare 10X dye solution by diluting DAPI to 100 ug/mL in fresh, complete culture medium. DAPI can be combined with other fluorescent probes, which should be diluted to 10 times the final desired concentration.
2. Without removing the medium from the cells, add 1/10 volume of 10X dye directly to the well.
3. Immediate mix thoroughly by gently pipetting the medium up and down. For larger well sizes (e.g., 24-well to 6-well plates), the plate can be gently swirled to mix.
4. Incubate cells at room temperature or 37°C for 5-15 minutes, then image. Note: Washing is not necessary for specific staining, but nuclear staining is stable after washing.

Staining of fixed cells or tissue sections

1. Dilute DAPI to 1 ug/mL in PBS. DAPI can be included together with antibodies or other probes, and can be diluted in buffers with detergent or blocking agents if convenient.
2. Add the PBS with dye to cells or tissue sections and incubate at room temperature for at least 5 minutes.
3. Image the samples; washing is optional but not required.
   Note: Samples can be stored at 4°C after staining and before imaging. Note: DAPI can be included directly in antifade mounting medium for one- step mounting and staining. When using DAPI in mounting medium, longer incubation times may be required for DAPI to completely penetrate the cell nuclei.

Staining bacteria or yeast

DAPI stains bacteria more dimly than mammalian cells. Live or killed bacteria can be stained with 10 ug/mL DAPI in PBS or 150 mM NaCl for 30 minutes at room temperature. DAPI tends to stain dead cells more brightly than live cells.

In *S. cerevisiae*, DAPI preferentially stains dead yeast with nuclear and cytoplasmic staining when used at 10 ug/mL in PBS; in live yeast DAPI shows dim mitochondrial staining.