Technical support: support@abbkine.com

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## **DAPI**

Cat #: BMD0063 Size: 10 mg/100 mg

| FQ  | DAPI   |     |                                     |  |  |
|-----|--|-----|-------------------------------------|--|--|
| REF | Cat #: BMD0063   | LOT | Lot #: Refer to product label       |  |  |
|     | Application range: Nuclear staining reagents for               |     | Recommended working concentrations: |  |  |
|     | DNA staining   |     | 0.5-10 μg/mL                        |  |  |
|     | Excitation/Emission wavelengths: Ex/Em (bound DNA) =360/460 nm |     |                                     |  |  |
| Å   | Storage: Stored at -20°C for 12 months, protected from light   |     |                                     |  |  |

## **Assay Principle**

DAPI has the molecular formula  $C_{16}H_{17}Cl_2N_5$ , molecular weight 350.3 and CAS number 28718-90-3. It is a nuclear staining reagent that can stain DNA. After embedding double-stranded DNA, DAPI releases blue fluorescence and its brightness is enhanced by about 20 times. DAPI is often used for apoptosis detection, which is observed by fluorescence microscopy or flow cytometry after staining. DAPI has a high photobleaching tolerance level and can be used to detect yeast mitochondrial DNA, chloroplast DNA, viral DNA and chromosome DNA. At lower concentrations (1  $\mu$ g/mL), DAPI is impermeable to living cells but can be used as a nuclear stain to fix cell or tissue parts. At higher concentrations (10  $\mu$ g/mL), DAPI can be used to stain living cells. In the case of adherent cells (96-well plates), 100  $\mu$ L of the staining solution was used in each well, and the concentration of the staining solution was calculated as 5  $\mu$ g/mL. 10 mg of the staining solution could be used in 20,000 wells.

$$\begin{array}{c|c} \text{2HCl} \\ \text{H}_2\text{N} & \begin{array}{c} \text{NH} \\ \text{N} \\ \end{array} \\ \begin{array}{c} \text{DAPI} \end{array} \end{array}$$

Figure 1. Molecular diagram

# **Materials Supplied and Storage Conditions**

| Kit components | Size  |        | Storage conditions          |
|----------------|-------|--------|-----------------------------|
| DAPI           | 10 mg | 100 mg | -20°C, protected from light |



### **Materials Required but Not Supplied**

· Fluorescent microscope, precision pipettes, disposable pipette tips, deionized water, PBS

## **Assay Procedure**

Note: For cell or tissue samples, after fixation, the fixative was removed by appropriate washing. If immunofluorescence staining was to be performed, DAPI staining was performed after the completion of staining. If no other staining was required, subsequent DAPI staining was performed directly.

1. DAPI was dissolved in deionized water to prepare 1 mg/mL DAPI aqueous solution, which was stored at -20°C, protected from light.

Note: DAPI cannot be dissolved directly with a buffer solution such as PBS; it needs to be dissolved with water first.

- 2. Appropriate amount of DAPI aqueous solution was added to PBS to prepare 5 µg/mL DAPI solution.
- 3. For adherent cells (the medium in the well plate was removed) or tissue sections, a small amount of DAPI staining solution was added to cover the sample. For suspended cells, at least three times the volume of the sample to be stained was added and mixed.
- 4. Cells were incubated at room temperature for 10-20 min.
- 5. Cells were washed twice with PBS or appropriate buffer for 3-5 min each time, and 50  $\mu$ L PBS was added to prevent cells from drying out after completion of washing.
- 6. Cells were observed using a fluorescence microscope with a filter with an excitation wavelength of 360 nm and an emission wavelength of 460 nm.

#### **Precautions**

- 1. Please immediately centrifugal the product to the bottom of the tube before use, and then conduct the subsequent experiments.
- 2. Compared with staining bacteria, DAPI dye is more sensitive for staining mammalian cells. When staining dead or alive bacteria, it is recommended to dissolve the staining solution in PBS or 150 mM NaCl into a final concentration of 10  $\mu$ g/mL, and staining for 30 min at room temperature. The staining of dead cells is usually brighter than that of living cells.
- 3. The recommended working concentration of DAPI for nuclear staining is 0.5-10 µg/mL.
- 4. DAPI is harmful to human body, please wear a laboratory coat and disposable gloves to operate, pay attention to appropriate protection.
- 5. Fluorescent dyes all have quenching problems, please try to avoid light to slow down the quenching.

#### **Disclaimer**

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.

