Title: DAPI (4',6-Diamidino-2-Phenylindole, Dihydrochloride) Nucleic Acid Fluorescent Staining

Approvals:
Preparer: W. H. Woodruff Date 13 May 2014
Reviewer: Maggie Bryans Date 12 July 2016

1. Purpose: This SOP is intended to provide the necessary instructions to stain cellular nuclei for fluorescent viewing.

2. Scope: These instructions will enable any qualified technician to properly prepare and stain the nuclei of many different adherent cell lines with the fluorescent stain, DAPI.

3. Responsibilities:
   3.1. It is the responsibility of the course instructor /lab assistant to ensure that this SOP is performed as directed and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.

4. References:
   4.1. SOP: ID# REA-004 Preparation of Dulbecco's Phosphate Buffered Saline
   4.2. SOP: ID# REA- 027 Preparation of DAPI (4',6-Diamidino-2-Phenylindole, Dihydrochloride) Nucleic Acid Fluorescent Stain, 5 mg/ml stock solution
   4.3. SOP: ID# BTC-011 Set Up and Operation of the Olympus Inverted Microscope for Cell Viewing Using Fluorescence
   4.4. SOP: ID# BTC-012 Set Up and Operation of the EVOS Inverted Microscope for Cell Viewing Using Fluorescence
   4.5. SOP: ID# TEC- 025 Paraformaldehyde Fixation of Adherent Cells in Culture

5. Definitions:
   5.1. Fluorescence: the ability of certain molecules to absorb energy at a specific wavelength and re-emit the energy at a longer wavelength (i.e., lower energy) as visible light.

6. Precautions:
   6.1. DAPI is a known mutagen and should be handled with care. The dye must be disposed of safely and in accordance with applicable local regulations.
   6.2. All appropriate PPE **must** be worn - lab. coat, gloves, goggles.

7. Materials:
   7.1. ~80 - 90% confluent cell culture in the wells of a 12- or 24-well plate, fixed as required.

7.2. DAPI (4',6-Diamidino-2-Phenylindole, Dihydrochloride) Nucleic Acid Fluorescent Stain, 5 mg/ml stock solution (14.3 mM)
7.3. Dubecco's Phosphate Buffered Saline (D-PBS), pH 7.2 - 7.4
7.4. 2 and 5 ml sterile serological pipettes
7.5. Waste beaker

8. Procedure:
8.1. General Preparations
8.1.1. Gather and inventory all the required materials.
NOTE: This protocol does not require aseptic techniques, therefore, you will not need to prep the Laminar Flow Safety Cabinet.

8.2. Sample Preparation
8.2.1. **NOTE:** Use the fixation protocol appropriate for your sample. DAPI staining is normally performed after all other staining. Note that fixation and permeabilization of the sample are not necessary for counterstaining with DAPI.
8.2.2. Equilibrate the cell sample 3 - 5 minutes in D-PBS

8.3. Dilute the 5 mg/ml DAPI stock solution (14.3 mM) to 300 nM in D-PBS
8.3.1. E.g., to prepare 10 ml of working solution
8.3.1.1. Measure 10 ml of D-PBS into an appropriate vessel
8.3.1.2. Add 0.2 ml (200 ul) of the stock DAPI solution to the D-PBS
8.3.1.3. Mix thoroughly

8.3.2. Add 300 - 500 ul of the diluted DAPI to the cell sample in each well, making sure the cells are completely covered

8.3.3. Incubate for 1 - 5 minutes

8.3.4. Drain and rinse the cell sample several times with D-PBS.

8.3.5. Initially, leave ~ 0.5 ml of D-PBS on the cells.

8.3.6. View the sample using a fluorescence microscope with appropriate filters.

9. History:

Name	Date	Amendment
W.H.Woodruff	13 May, 2014	Initial release
W.H. Woodruff	13 July, 2016	Minor updates to volumes, precautions.

NOTE: The following information is not usually found on an SOP but because this is a teaching instrument, I have included it here for completeness.

Background:

The blue-fluorescent DAPI nucleic acid stain preferentially stains dsDNA; it appears to associate with AT clusters in the minor groove. Binding of DAPI to dsDNA produces a ~20-fold fluorescence enhancement, apparently due to the displacement of water molecules from both DAPI and the minor groove. DAPI also binds RNA, however in a different binding mode—one thought to involve AU-selective intercalation. The DAPI/RNA complex exhibits a longer-wavelength fluorescence emission maximum than the DAPI/dsDNA complex (~500 nm versus ~460 nm) and a quantum yield that is only about 20% as high.

DAPI is a popular nuclear counterstain for use in multicolor fluorescent techniques. Its blue fluorescence stands out in vivid contrast to green, yellow, or red fluorescent probes of other structures. When used according to this protocol, DAPI stains nuclei specifically, with little or no cytoplasmic labeling. Both DAPI and DAPI dilactate work well in this protocol. The DAPI dilactate form may be somewhat more water soluble. The counterstaining protocols are compatible with a wide range of cytological labeling techniques—direct or indirect antibody-based detection methods, mRNA in situ hybridization, or staining with fluorescent reagents specific for cellular structures. DAPI can also serve to fluorescently label cells for analysis in multicolor flow cytometry experiments. The protocols can be modified for tissue staining or for staining unfixed cells or tissues.

Fluorescence Spectral Characteristics

The excitation maximum for DAPI bound to dsDNA is 358 nm, and the emission maximum is 461 nm. DAPI can be excited with a xenon or mercury-arc lamp or with a UV laser. DAPI may be used in flow cytometry systems utilizing UV excitation sources.