For life science research only. Not for use in diagnostic procedures.

DAPI

4’,6-Diamidine-2’-phenylindole dihydrochloride

Version: 19

Content Version: March 2021

Cat. No. 10 236 276 001 10 mg

Store product at +15 to +25°C.

1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Content
1	DAPI	Crystallized powder	1 bottle, 10 mg

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +15 to +25°C, the product is stable through the expiration date printed on the label.

Vial / Bottle	Label	Storage
1	DAPI	Store at +15 to +25°C. ⚠ ***Keep protected from light.***

Storage Conditions (Working Solution)

Working Solution	Concentration	Storage and Stability
DAPI stock solution	1 to 5 mg/ml	Store at −15 to −25°C for 12 months.
Working solution	1 µg/ml	Store at +2 to +8°C for approximately 6 months.

Reconstitution

Reconstitute DAPI in 2 to 10 ml double-distilled water to a final concentration of 1 to 5 mg/ml.
⚠ ***Store in aliquots at −15 to −25°C.***

1.3. Additional Equipment and Reagent required

For preparation of DAPI working solution

- Methanol

For staining of monlayer cultures

- Methanol
- Glycerol or PBS
- Fluorescence miscroscope

For staining of suspension cultures

- PBS
- Fluorescence miscroscope

Permanent preparations

- Methanol
- PBS
- Anti-fading mounting medium, such as glycerol/PBS (1:10) containing 2 to 7 mM 4-phenylenediamine, pH 8.5 to 9.0

1.4. Application

DAPI is used for the detection of mycoplasma infections in cell cultures.

2. How to Use this Product

2.1. Before you Begin

General Considerations

Detection techniques

A variety of techniques have been developed for the detection of cell culture mycoplasmas:
- DNA staining
- Mycoplasma-mediated cytotoxicity
- Biochemical detection methods
- Electron microscopy
- PCR (Mycoplasma PCR ELISA*)

DNA staining employing fluorescent dyes that bind specifically to DNA is the most popular method. This method is quick and simple to perform. Two dyes, 4′,6-diamidine-2′-phenylindole (DAPI) and bisbenzimide (H33258) have been widely used. The rationale behind this assay is that mycoplasma-free cultures exhibit only nuclear fluorescence. Mycoplasma-infected cultures also display extranuclear fluorescence. Mitochondrial DNA is not apparent in preparations stained either with DAPI or H33258.

Working Solution

Preparation of DAPI stock solution

Dissolve crystallized powder in double-distilled water to a final concentration of 1 to 5 mg/ml. Solubility in water is 25 mg/ml.
⚠ ***Do not use any buffers.***

Preparation of working solution

Dilute the stock solution with methanol to a final concentration of 1 µg/ml.
⚠ ***Store working solution at +2 to +8°C for approximately 6 months.***

2.2. Protocols

Staining of monolayer cultures

Before the assay, pass cell cultures in antibiotic-free media for a minimum of two passages. The cultures should be assayed 3 to 4 days after passage. The cell supernatant will contain 1 × 10^7 to 1 × 10^8 CFU/ml; additional organisms are adsorbed onto host cells.
ⓘ *See section,* **Working Solution** *for additional information on preparing solutions.*

1. Allow cultures to reach 50 to 70% confluence.
   ⓘ *Allowing cultures to reach confluence will impair subsequent visualization of mycoplasmas. Cultures may be grown on coverslips in petri dishes.*
2. Pour off the medium from the cells.
3. Wash once with DAPI-methanol (working solution, 1 µg/ml).
4. Cover the cells with DAPI-methanol and incubate for 15 minutes at +37°C.
5. Pour off the staining solution.

6. Wash once with methanol.

7. Place the inverted coverslip on a microscope slide using glycerol or PBS as mounting medium; avoid water.

8. Examine under a fluorescence microscope with 340/380 nm excitation filter and LP 430 nm barrier filter, such as Leitz filter combination: BP 340-380, RKB 400, LP 430; Zeiss filter combination: BP 365/11, FT 395, LP 397, or BP 340-380, RKP 400, LP 430.
   A total of 500× (40 × 12.5) magnification is generally sufficient in detecting brightly fluorescent mycoplasmas. However, best results are obtained using a 100× oil immersion objective.

Staining of suspension cultures

See section, **Working Solution** *for additional information on preparing solutions.*

1. Spin the cells down.

2. Pour off the supernatant.

3. Wash once in DAPI-methanol.

4. Suspend the cells in DAPI-methanol (working solution, 1 µg/ml) for 15 minutes at +37°C.

5. Spin the cells down.

6. Pour off the staining solution.

7. Add PBS just to suspend the cells.

8. Place one drop on a microscope slide, cover with a coverslip, and examine under a fluorescence microscope.

Permanent preparations

1. Stain as described in section, **Staining of suspension cultures**.

2. Pour off the staining solution.

3. Wash once with methanol and air dry.

4. Embed the preparation with a suitable anti-fading mounting medium, such as glycerol/PBS (10:1) containing 2 to 7 mM 4-phenylenediamine, pH 8.5 to 9.0.

2.3. Parameters

Absorbance

Absorbance maximum in aqueous solution: λ = 340 nm

Chemical Formula

$C_{16}H_{15}N_5 \times 2\ HCl$

Emission

Emission maximum in aqueous solution: λ = 488 nm

Molecular Weight

350.3 Da

Purity

≥90% (from N)

3. Results

An uncontaminated cell culture shows only nuclear fluorescence against a dark cytoplasmic background. Mitochondrial DNA does bind the fluorochrome, but at levels imperceptible by routine fluorescent microscopy. Mycoplasmas, however, which have approximately 10 times the DNA content of mitochondria, are readily detected as bright foci against the dark background. They give pin points over the cytoplasm and sometimes in intercellular spaces (Fig. 1). Not all of the cells will necessarily be infected, so most of the preparation should be carefully scanned before declaring the culture uncontaminated.

To overcome problems associated with the analysis of many different cells, to detect low-level contaminations in resistant cell lines, and to screen potentially infected sera, it is recommended to use an indicator cell such as 3T6 mouse embryo fibroblasts, Vero monkey cells, or Mv1Lu mink lung cells. Specimens to be analyzed are inoculated into the indicator cell culture and, after an appropriate incubation period, the indicator cell line is analyzed for the presence of mycoplasmas.

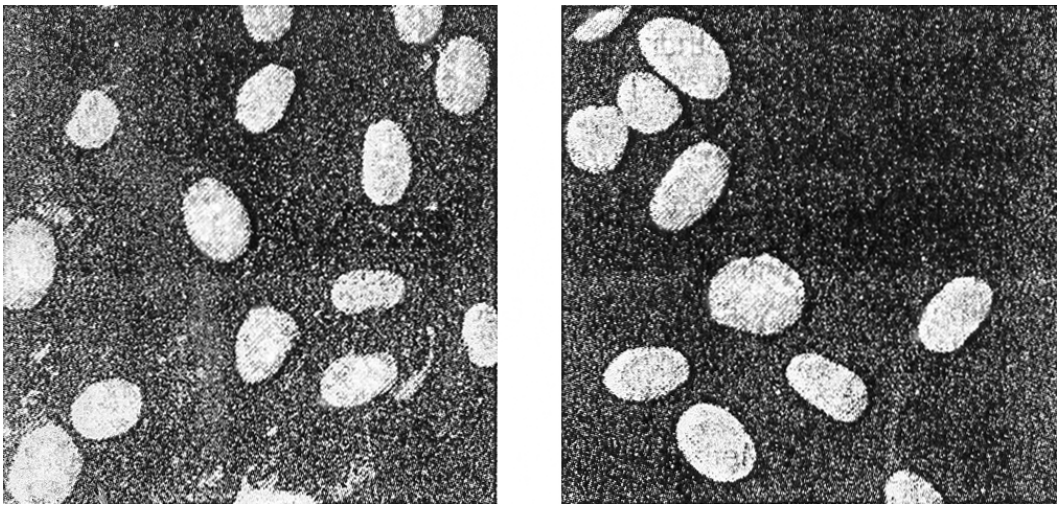

Fig. 1: Fibroblast cell line L-929 after DAPI staining of DNA.
A: Cell culture contaminated with mycoplasmas.
B: Complete absence of mycoplasmas after a 3-cycle treatment with BM-Cyclin*(courtesy of Dr. J. Schmidt, Munich-Neuherberg).

4. Additional Information on this Product

4.1. Test Principle

The fluorescent dye DAPI binds selectively to DNA and forms strongly fluorescent DNA-DAPI complexes with high specificity. When adding DAPI to tissue culture cells, it is rapidly taken up into cellular DNA yielding highly fluorescent nuclei and no detectable cytoplasmic fluorescence. If the cells are contaminated with mycoplasmas, characteristic discrete fluorescent foci are readily detected over the cytoplasm and sometimes in intercellular spaces.

Statistics on the incidence of mycoplasma infections of cell cultures range from 1 to 92%. The origins of mycoplasma infection of cell cultures include:

- Bovine serum (*A. laidlawii, M. arginini, M. hyorhinis*)
- Laboratory personnel (*M. orale*)
- Mycoplasma-infected cultures

Mycoplasmas produce various effects on the infected cell culture. Mycoplasma infection cannot be detected by naked eye other than by signs of deterioration in the culture. It is important to recognize that mycoplasmas do not always reveal their presence with macroscopic alterations of the cells or media. Many mycoplasma contaminants, particularly in continuous cell lines, grow slowly and do not destroy host cells. Therefore, there is an absolute requirement for routine, periodic assays for possible covert contamination of all cell cultures, particularly continuous or established cell lines.

5. Supplementary Information

5.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

5.2. Changes to previous version

Layout changes.
Editorial changes.

5.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
BM-Cyclin	37.5 mg, for 2 x 2.5 l medium	10 799 050 001

5.4. Trademarks

All product names and trademarks are the property of their respective owners.

5.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

5.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

5.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

5.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

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