

4',6-Diamidine-2'-phenylindole dihydrochloride (DAPI)

crystallized, nonsterile

10 mg, Cat. No. 236276

Product description

Formula: $C_{16}H_{15}N_5 \cdot 2HCl$; M_r 350.3.

Typical analysis: >90% (from N).

Formulation: Powder (crystallized); nonsterile.

Reconstitution: In 2–10 ml water; 1–5 mg/ml final concentration; solubility in water: 25 mg/ml.

Stability: stable at room temperature, protected from light. The reconstituted solution is stable at $-20^\circ C$ for 1 year.

Application

Figures on the incidence of mycoplasmal infections of cell cultures range from 1–92% (1–4). The origins of mycoplasmal infection of cell cultures are bovine serum (*A. laidlawii*, *M. arginini*, *M. hyorhinis*), laboratory personnel (*M. orale*) and mycoplasma-infected cultures. Mycoplasmas produce various effects on the infected cell culture (2–4). Mycoplasmal infection cannot be detected by naked eye other than by signs of deterioration in the culture. It is important to appreciate 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. Prior to this assay cell cultures should be passaged in antibiotic-free media for a minimum of two passages. The cultures should be assayed 3–4 days after passage. The cell supernatant will contain 10^7 – 10^8 CFU/ml, additional organisms are adsorbed onto host cells.

A variety of techniques have been developed for the detection of cell culture mycoplasmas (e.g. DNA staining, mycoplasma-mediated cytotoxicity, biochemical detection methods, electron microscopy) (2,3). 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-diamidino-2-phenylindole (DAPI) and bisbenzimidazole (H33258)* have been widely used (5–7). 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 instruction

Assay principle

The fluorescent dye DAPI binds selectively to DNA and forms strongly fluorescent DNA-DAPI complexes with high specificity. DAPI has in aqueous solution an absorbance maximum at $\lambda = 340$ nm and an emission maximum at $\lambda = 488$ nm. On 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.

Preparation of stock solution

- Dissolve in water to a final concentration of 1–5 mg/ml. Do not use any buffers.
- This stock solution can be stored at $-20^\circ C$. It is recommended to prepare appropriate aliquots.

Preparation of working solution

- Dilute the stock solution with methanol to a final concentration of 1 $\mu g/ml$. The working solution is stable at $+4^\circ C$, for about 6 months.

Staining of monolayer cultures

- Allow cultures to reach 50–70% confluence. Allowing cultures to reach confluence will impair subsequent visualisation of mycoplasmas. Cultures may be grown on coverslips in petri dishes.
- Pour off the medium from the cells.
- Wash once with DAPI-methanol (working solution, 1 $\mu g/ml$).
- Cover the cells with DAPI-methanol and incubate for 15 min at $37^\circ C$.
- Pour off the staining solution.
- Wash once with methanol.

- Place the inverted coverslip on a microscope slide, using glycerol or PBS as mounting medium, avoid water. Examine under a fluorescence microscope with 340/380 nm excitation filter and LP 430 nm barrier filter (e. g. 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. But best results are obtained using a 100 × oil immersion objective.

Staining of suspension cultures

- Spin the cells down.
- Pour off the supernatant.
- Wash once in DAPI-methanol.
- Suspended the cells in DAPI-methanol (working solution, 1 µg/ml) and incubate for 15 min at 37 °C.
- Spin the cells down.
- Pour off the staining solution.
- Add PBS just to suspend the cells.
- Place one drop on a microscope slide, cover with a coverslip and examine under a fluorescence microscope.

Permanent preparations

- Stain as above.
- Pour off the staining solution.
- Wash once with methanol.
- Air dry.
- Embed the preparation with a suitable anti-fading mounting medium [e. g. glycerol/PBS (10:1) containing 4-phenylenediamine, 2-7 mmol/l, pH 8.5-9.0 (8)].

Analysis

An uncontaminated cell culture shows only nuclear fluorescence against a dark cytoplasmic background. Mitochondrial DNA does not 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 (s. 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 (9). Specimens to be analysed are inoculated into the indicator cell culture and, after an appropriate incubation period, the indicator cell line is analysed for the presence of mycoplasmas.

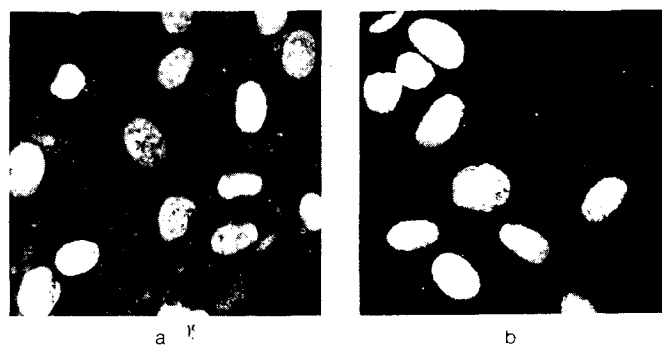


Fig. 1
Fibroblast cell line L-929 after DAPI staining of DNA.
a: cell culture contaminated with mycoplasmas;
b: complete absence of mycoplasmas after a 3 cycle treatment with BM-Cyclin* (by courtesy of by Dr. J. Schmidt, Munich-Neuherberg).

References

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*available from Boehringer Mannheim GmbH