For life science research only. Not for use in diagnostic procedures.
FOR *IN VITRO* USE ONLY.

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

Powder, non sterile

Cat. No. 236 276

10 mg

Version 4, August 2001

Store this kit at 15–25°C

1. Product overview

Formulation

Powder (crystallized); non sterile

Properties

Formula	$C_{16}H_{15}N_5 \times 2$ HCl
Molecular weight	M_r 350.3
Solubility in water	25 mg/ml
Absorbance maximum in aqueous solution	$\lambda = 340$ nm
Emission maximum in aqueous solution	$\lambda = 488$ nm

Typical analysis

>90% (from N.)

Application

Detection of mycoplasmal infections of cell cultures.

Assay principle

The fluorescent dye DAPI binds selectively to DNA and forms strongly fluorescent DNA-DAPI complexes with high specificity.

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.

Reconstitution

In 2–10 ml double dist. water; 1–5 mg/ml final concentration.

Note: Prepare aliquots and store at −15 to −25°C.

Storage stability

Stable at 15–25°C, protected from light, until the expiration date printed on the label.

Solution	Storage/stability
Stock solution (1–5 mg/ml)	−15 to −25°C for 12 months
Working solution (1µg/ml)	2–8°C for about 6 months

Background information

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 detoriation 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.

Detection techniques

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,
- ELISA (Mycoplasma Detection Kit*) or by PCR (Mycoplasma PCR ELISA*) (2, 3, 5).

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 (6–8). 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.

2. Procedures and required materials

2.1 Before you begin

General considerations

Prior to the assay cell cultures should be passed 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 organism are adsorbed onto host cells.

Preparation of stock solution

Dissolve in double dist. water to a final concentration of 1–5 mg/ml.
Note: Do not use any buffers.

Preparation of working solution

Dilute the stock solution with methanol to a final concentration of 1 µg/ml. The working solution is stable at 2–8°C, for about 6 months.

2.2 Staining of monolayer cultures

Procedure

Please find the protocol for the staining of monolayer cultures in the following table.

Step	Action
1	Allow cultures to reach 50–70% confluence. ***Note***: 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 min 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. 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.

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2.3 Staining of suspension cultures

Procedure

Please find the protocol for the staining of suspension cultures in the following table.

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

2.4 Permanent preparations

Procedure

Please refer to the following table.

Step	Action
1	Stain as described in 2.3.
2	Pour off the staining solution.
3	Wash once with methanol.
4	Air dry.
5	Embed the preparation with a suitable anti-fading mounting medium [e.g. glycerol/PBS (10:1) containing 2-7 mM 4-phenylenediamine, pH 8.5–9.0 (9)].

3. Analysis

General

An uncontaminated cell culture shows only nuclear fluorescence against a dark cytoplasmatic 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 (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 (10). 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.

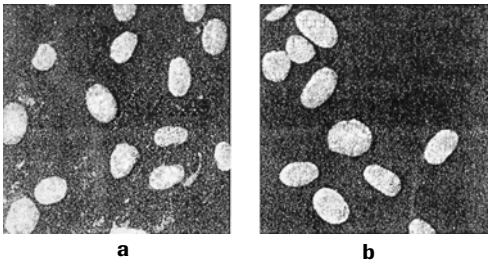
Figure 1

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).



a b

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5. Related products

Product	Pack Size	Cat. No.
BM-Cyclin Antibiotic combination for the elimination of mycoplasma from cell cultures	37.5 mg (for 2 × 2.5 l culture medium)	799 050
Mycoplasma Detection Kit (Enzyme Immunoassay)	1 kit (25 tests)	1 296 744
Mycoplasma PCR ELISA	1 kit (96 reactions)	1 663 925

*available from Roche Molecular Biochemicals

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