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

Roche

BM Cyclin

Cat. No. 10 799 050 001

37.5 mg (for 2 × 2.5 l medium)

Version 16.0
Content version: October 2010

Store at +2 to +8°C

1. What this Product Does

Kit Contents

Vial/Cap	Label	Contents
1	BM Cyclin 1	• 25 mg, lyophilized • pleuromutilin derivative
2	BM Cyclin 2	• 12.5 mg, lyophilized • tetracycline derivative

Storage and Stability

Lyophilized stable at +2 to +8°C until the expiration date printed on the label.

⚠ Reconstituted stable at −15 to −25°C for at least 6 months.

Additional Equipment and Reagents Required

DAPI*

Application

BM Cyclin is used for the elimination of mycoplasma from infected cell cultures without marked cytotoxic side effects.

Sensitive Organisms:

Mycoplasmas and bacteria

Only BM Cyclin was found to effectively eliminate

- Acholesplasma laidlawii,
- Mycoplasma arginini,
- Mycoplasma hyorhinis, and
- Mycoplasma orale

from experimentally contaminated and chronically infected cell lines.

These mycoplasma strains account for more than 85% of the contaminations in animal cell cultures (1).

2. How To Use this Product

2.1 Before You Begin

Preparation of Stock Solution

Dissolve content of BM Cyclin 1 and BM Cyclin 2 in 10 ml sterile PBS or water.

These are 250× concentrated stock solutions and can be stored at −15 to −25°C for at least 6 months.

Recommended Working Concentration

BM Cyclin 1: 10 μg/ml.

BM Cyclin 2: 5 μg/ml.

These concentration do not affect the growth of most cells. For sensitive cell lines lower concentrations may be used.

2.2 Protocol

Treatment of Mycoplasma-contaminated Cell Cultures

1. Remove culture medium from culture vessels by aspiration.
2. Add new medium containing BM Cyclin 1 (4 μl of stock solution/ml, final concentration 10 μg/ml).
3. Cultivate the cells for 3 days in the usual way
4. Remove culture medium.
5. Add new culture medium containing BM Cyclin 2 (4 μl of stock solution/ml, final concentration 5 μg/ml).
6. Cultivate the cells for 4 days.
7. Repeat the cycle twice.
8. Check for mycoplasma contamination (*e.g.,* with a DNA fluorochrome such as DAPI).

2.3 Detection of Mycoplasmas with DAPI

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 DAPI Stock Solution

Dissolve DAPI in water to a final concentration of 1– 5 mg/ml.

⚠ Do not use any buffers.

This stock solution can be stored at −15 to −25°C. It is recommended to prepare appropriate aliquots.

Preparation of DAPI Working Solution

Dilute the stock solution with methanol to a final concentration of 1 μg/ml.

Protocol for Staining of Monolayer Cultures

1. Allow cultures to reach 50–70% 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 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.

8	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.

Protocol for Staining of Suspension Cultures

1	Spin the cells down.
2	Pour off the supernatant.
3	Suspended the cells in DAPI-methanol (working solution, 1 µg/ml) for 15 min at 37°C.
4	Spin the cells down.
5	Pour off 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.

Protocol for Permanent Preparations

1	Stain as above.
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

2.4 Analysis

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 (see Fig. 1). Not all of the cells will necessarily be infected, so most of the preparation should be 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 (2). 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.

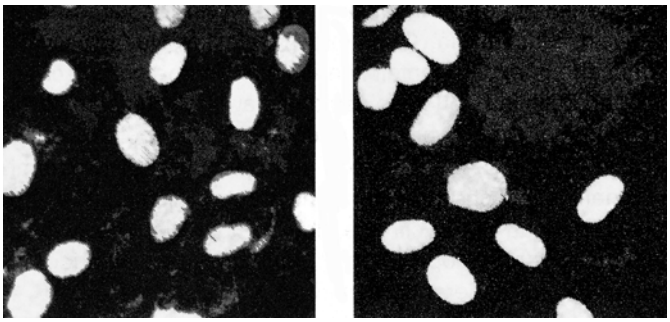

Fig. 1: Fibroblast cell line L-929 after DAPI staining of DNA.
a: untreated cell culture shows mycoplasma contamination;
b: complete absence of mycoplasma contamination after a 3 cycle treatment with BM Cyclin (by courtesy of by Dr. J. Schmidt, Munich-Neuherberg).

3. Additional Information on this Product

Test Principle for the Elimination of Mycoplasma with BM Cyclin

Treatment of mycoplasma-contaminated cell cultures with the antibiotic combination BM Cyclin leads to an effective elimination of the mycoplasmas. Treatment with BM Cyclin includes three or more cycles. Each cycle grows cells for 3 days in BM Cyclin 1 alternating with 4 days in BM Cyclin 2 containing medium.

It is recommended to check the culture every week (*e.g.,* with a DNA fluorochrome such as DAPI) for the presence of mycoplasmas (Fig. 1). Normally mycoplasmas will be absent after the second cycle (week) but as this is only an optical determination the treatment should be continued for one more cycle. This is important for treatment of sensitive cells with a lower BM Cyclin concentration because here the removal of the mycoplasma contamination will take longer *e.g.,* 3 cycles instead of 2. In any case it is recommended to employ an extra cycle after absence of contamination is first seen.

Background Information

The contamination of tissue cultures by mycoplasmas remains one of the major problems encountered in biological research using cultured cells. Mycoplasmas can produce extensive changes in cultures they infect.

These organisms are resistant to many of the antibiotics that are in common use in cell cultures (3–4).

This problem has been highlighted by the introduction of sensitive, quick methods for the detection of cell culture mycoplasmas, such as the DNA fluorescent staining method using fluorochromes such as DAPI* or Hoechst 33258 (bisbenzimide)* (5–7).

The efficiency of BM Cyclin has been tested in comparison with several other antibiotics (kanamycin, tylosine, spectinomycin, lincomycin, gentamicin).

BM Cyclin has been reported to be the method of choice for the elimination of mycoplasmas from contaminated hybridomas (8) and other cell lines (9). The antibiotic combination BM Cyclin is used for the elimination of mycoplasmas from infected cell cultures. It is recommended to use BM Cyclin only for elimination of mycoplasmas and not for prevention of contamination. Also a simultaneous use of BM Cyclin 1 and 2 together is to be avoided. When compared with other mycoplasma elimination procedures the BM Cyclin treatment has the advantage of effectiveness combined with the absence of adverse effects.

References

1 Barile M F *et al.* Mycoplasma Infection of Cell Cultures (McGarrity, G. J., Murphy, D. G. & Nichols, W. W., eds.) Plenum Press, New York (1978), pp.35–45.
2 McGarrity G J. Advances in Cell Culture, Vol. 2 (Maramorosch, K., eds.) Academic Press, New York, (1982) pp.9 –131.
3 McGarrity G J & Kotani H. The Mycoplasmas, Vol. IV (Razin, S. & Barile, M. F., eds.) Academic Press, New York, (1985) pp.353–390.
4 McGarrity G J *et al. In Vitro* (1984);**20**:1–18.
5 Russel W C *et al. Nature* (1975);**253**:461–462.
6 Chen T R. *Exp. Cell. Res.* (1977);**104**:255–262.
7 Hessling J J *et al. J. Immunol.Methods* (1980);**38**:315–324.
8 Ravaoarinoro M & Lecomte J. *Hybridoma* (1988);**7**: 79–86.
9 Branch D R & Guilbert L J. *J. Tissue Cult. Meth.* (1986);**10**:101–108.
10 Krenik K D *et al. J. Immunol. Meth.* (1989);**117**: 91–97.
11 Darai G *et al. In Vitro* (1983);**19**: 7–15.
12 de Diesbach P *et al.* Infection by Mycoplasma hyorhinis strongly enhances uptake of antisense oligonucleotides: a reassessment of receptor-mediated endocytosis in the HepG2 cell line. *Nucleic Acids Research (*2003);**31**(3):886-892.
13 Jin-Ying Ning *et al.* Identification of antigens by monoclonal antibody PD4 and its expression in *Escherichia coli. World Journal of Gastroenterology* (2003); **9**(10):2164-2168.
14 Jung Hsuan *et al.* Detection and Treatment of Mycoplasma Contamination in Cultured Cells. *Chang Gung Med J Vol.* (2003);**26**(4):250–258.
15 Flanagan S A & Meckling K A. All-trans-retinoic acid increases cytotoxicity of 1-β-d-arabinofuranosylcytosine in NB4 cells. *Cancer Chemotherapy and Pharmacology* (2003);**51**(5):363-375.

4. Supplementary Information

4.1 Conventions

Text Conventions
To make information consistent and memorable, the following text conventions are used in this package insert:

Text Convention	Use
Numbered Instructions labeled ❶, ❷ etc.	Steps in a procedure that must be performed in the order listed
Asterisk *	Denotes a product available from Roche Applied Science

Symbols
In this package insert the following symbols are used to highlight important information:

Symbol	Description
	Information Note: Additional information about the current topic or procedure.
⚠	Important Note: Information critical to the success of the procedure or use of the product.

4.2 Ordering Information

Roche Applied Science offers a large selection of reagents and systems for life science research. For a complete overview of related products and manuals, please visit and bookmark our home page www.roche-applied-science.com.

Trademarks
All brands or product names are trademarks of their respective holders.

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

Contact and Support

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

www.roche-applied-science.com/support

To call, write, fax, or email us, visit the Roche Applied Science home page, www.roche-applied-science.com, and select your home country. Country-specific contact information will be displayed. Use the Product Search function to find Pack Inserts and Material Safety Data Sheets.

Roche Diagnostics GmbH
Roche Applied Science
68298 Mannheim
Germany