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

BM-Cyclin

Version: 19
Content Version: December 2020

Cat. No. 10 799 050 001 37.5 mg
for 2 x 2.5 l medium

Store lyophilizate at +2 to +8°C.

1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Content
1	BM-Cyclin 1	▪ Lyophilized ▪ Pleuromutilin derivative.	1 bottle, 25 mg
2	BM-Cyclin 2	▪ Lyophilized ▪ Tetracycline derivative.	1 bottle, 12.5 mg

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +2 to +8°C, the lyophilizate is stable through the expiration date printed on the label.

Vial / Bottle	Label	Storage
1	BM-Cyclin 1	Store at +2 to +8°C.
2	BM-Cyclin 2	

Storage Conditions (Working Solution)

Store the prepared 250× stock solutions at −15 to −25°C for at least 6 months.

1.3. Additional Equipment and Reagent required

For preparation of BM-Cyclin 1 and 2 stock solutions

- Sterile PBS
- Sterile, double-distilled water

For treatment of mycoplasma-contaminated cell cultures

- Cell culture medium
- DAPI*

For staining of monolayer cultures

- Methanol
- DAPI-methanol working solution, 1 µg/ml
  See section, **Working Solution** *for additional information on preparing solution.*
- Glycerol or PBS
- Fluorescence microscope

For staining of suspension cultures

- DAPI-methanol working solution, 1 µg/ml
  See section, **Working Solution** *for additional information on preparing solution.*
- PBS
- Fluorescence microscope

For permanent preparations

- Methanol
- DAPI-methanol working solution, 1 µg/ml
  See section, **Working Solution** *for additional information on preparing solution.*
- PBS
- Anti-fading mounting medium, such as glycerol/PBS (10:1) containing 2 to 7 mM 4-phenylenediamine, pH 8.5 to 9.0

1.4. Application

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

2. How to Use this Product

2.1. Before you Begin

Safety Information

Laboratory procedures

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis / Binding Buffer or take appropriate measures, according to local safety regulations.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.
- Wash hands thoroughly after handling samples and reagents.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online on dialog.roche.com, or upon request from the local Roche office.

Working Solution

Preparation of stock and working solutions

Solution	Preparation
BM-Cyclin 1 stock solution	To prepare a 250x stock solution, dissolve content of each in 10 ml sterile PBS or sterile, double-distilled water.
BM-Cyclin 2 stock solution	
DAPI stock solution	▪ Dissolve DAPI in sterile, double-distilled water to a final concentration of 1 to 5 mg/ml. ⚠ ***Do not dissolve in any buffers.*** ▪ Store in aliquots at −15 to −25°C.
DAPI working solution	Dilute the DAPI stock solution with methanol to a final concentration of 1 µg/ml.

2.2. Protocols

Treatment of mycoplasma-contaminated cell cultures

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

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.
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 with a DNA fluorochrome such as DAPI.

Detection of mycoplasmas with DAPI

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 λ = 340 nm and an emission maximum at λ = 488 nm. After 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.

Staining of monolayer cultures

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. Suspend the cells in DAPI-methanol (working solution, 1 µg/ml) for 15 minutes 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.

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

Specificity

Sensitive organisms

Mycoplasmas and bacteria are sensitive to BM-Cyclin.
Only BM-Cyclin was found to effectively eliminate the following from experimentally contaminated and chronically infected cell lines:

- *Acholesplasma laidlawii*
- *Mycoplasma arginini*
- *Mycoplasma hyorhinis*
- *Mycoplasma orale*

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

Working Concentration

BM-Cyclin 1: 10 µg/ml
BM-Cyclin 2: 5 µg/ml

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

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, therefore 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. 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.

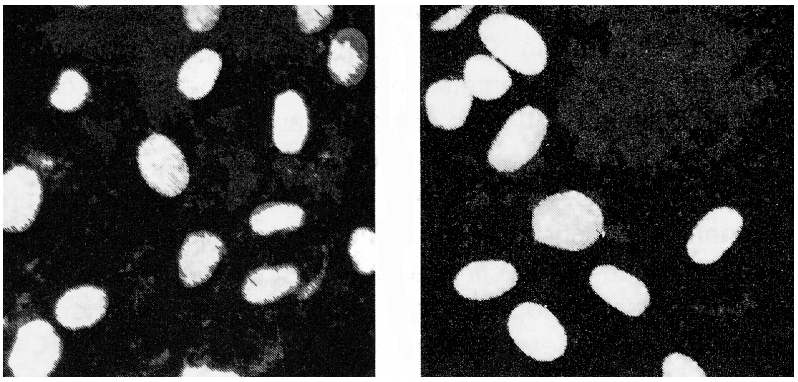

Fig. 1: Fibroblast cell line L-929 after DAPI staining of DNA.
A: Untreated cell culture shows mycoplasma contamination.
B: Complete absence of mycoplasma contamination after a 3-cycle treatment with BM-Cyclin (courtesy of Dr. J. Schmidt, Munich-Neuherberg).

4. Additional Information on this Product

4.1. Test Principle

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. 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). 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 and other cell lines. 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.

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 with a DNA fluorochrome such as DAPI for the presence of mycoplasmas, see **Figure 1, section, Results**. 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 like 3 cycles instead of 2. In any case, it is recommended to employ an extra cycle after absence of contamination is first seen.

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.
Update to include new safety Information to ensure handling according controlled conditions.

5.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
DAPI	10 mg	10 236 276 001

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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