BM Cyclin
Cat. No. 10 799 050 001
37.5 mg (for 2 × 2.5 l medium)

1. What this Product Does

Kit Contents

<table>
<thead>
<tr>
<th>Vial/Cap</th>
<th>Label</th>
<th>Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BM Cyclin 1</td>
<td>• 25 mg, lyophilized pleuromutilin derivative</td>
</tr>
<tr>
<td>2</td>
<td>BM Cyclin 2</td>
<td>• 12.5 mg, lyophilized tetracycline derivative</td>
</tr>
</tbody>
</table>

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.

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 λ = 340 nm and an emission maximum at λ = 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.
Protein for Permanent Preparations


2. 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-phenylendiamine, pH 8].

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 Hamster 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 culture cells. 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


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).
4. Supplementary Information

4.1 Conventions

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

<table>
<thead>
<tr>
<th>Text Convention</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Numbered Instructions</td>
<td>Steps in a procedure that must be performed in the order listed</td>
</tr>
<tr>
<td>Asterisk *</td>
<td>Denotes a product available from Roche Diagnostics</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>📖</td>
<td>Information Note: Additional information about the current topic or procedure.</td>
</tr>
<tr>
<td>⚠️</td>
<td>Important Note: Information critical to the success of the procedure or use of the product.</td>
</tr>
</tbody>
</table>

4.2 Changes to Previous Version

- Editorial changes

4.3 Ordering Information

<table>
<thead>
<tr>
<th>Product</th>
<th>Pack Size</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>X-tremeGENE 9 DNA Transfection</td>
<td>0.4 ml</td>
<td>06 365 779 001</td>
</tr>
<tr>
<td>Reagent</td>
<td>1 ml</td>
<td>06 365 787 001</td>
</tr>
<tr>
<td></td>
<td>5 × 1 ml</td>
<td>06 365 809 001</td>
</tr>
<tr>
<td>X-tremeGENE HP DNA Transfection</td>
<td>0.4 ml</td>
<td>06 366 244 001</td>
</tr>
<tr>
<td>Reagent</td>
<td>1 ml</td>
<td>06 366 236 001</td>
</tr>
<tr>
<td></td>
<td>5 × 1 ml</td>
<td>06 366 546 001</td>
</tr>
<tr>
<td>DAPI</td>
<td>10 mg</td>
<td>10 236 276 001</td>
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</tbody>
</table>

4.4 Trademarks

X-TREMEGENE is a trademark of Roche. All other product names and trademarks are the property of their respective owners.

4.5 Regulatory Disclaimer

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

4.6 Disclaimer of License

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

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