Phleomycin Powder

Selective antibiotic for the Sh ble gene; cell culture tested
Catalog code: ant-ph-2p, ant-ph-5p, ant-ph-10p

For research use only
Version 18C26-MM

PRODUCT INFORMATION

Content
Phleomycin is supplied as a blue powder.
• ant-ph-2p: 250 mg
• ant-ph-5p: 2 x 250 mg (500 mg)
• ant-ph-10p: 1 g

Note: As a powder, phleomycin is very hygroscopic. Keep tubes tightly closed after each use and protect from moisture.

Storage and stability
- Phleomycin is shipped at room temperature. Store phleomycin powder at 4 °C. The expiry date is specified on the product label.
- Store phleomycin solutions at 4 °C or -20 °C. Do not use phleomycin solutions after the expiry date on the product label. Avoid repeated freeze-thaw cycles.
- Phleomycin is sensitive to high concentrations of acids but a short-term exposure to dilute acids can be tolerated.

QUALITY CONTROL

Each lot is thoroughly tested to ensure the absence of lot-to-lot variation.
- Endotoxin level: < 1 EU/mg
- Physicochemical characterization (including HPLC, pH, appearance)
- Cell culture tested: potency validated in phleomycin-sensitive and phleomycin-resistant bacterial, fungal and mammalian cells
- Non-cytotoxicity of trace contaminants: absence of long-term effects confirmed in phleomycin-resistant cells

BACKGROUND

Phleomycin is a selection antibiotic that is active against most bacteria, filamentous fungi, yeast, plant and animal cells. Use of phleomycin is recommended for cells poorly sensitive to Zeocin™, i.e. filamentous fungi and yeast. Phleomycin resistance is conferred by the Sh ble gene; cell culture tested

PHYSICAL PROPERTIES

CHEMICAL PROPERTIES

Phleomycin is a mixture of structurally related antibiotics which differ by their terminal amine residues. They are in a copper chelated form giving the solution a blue color. The information below refers to Phleomycin D1.

CAS number: 11006-33-0
Empirical formula: C55H85O21N20S2Cu, HCl
Molecular weight: 1525

METHOD

Preparation of phleomycin solution
1. Resuspend phleomycin in HEPES buffer (5 g/l, pH 7.2 +/- 0.1) at a concentration of 20 mg/ml.
2. Sterile filter the solution using a 0.22 µm sterile filter.
3. Store at 4 °C for 12 months or -20 °C for 18 months.

CONDITIONS OF SELECTION

Most cells growing aerobically are killed by 0.1 to 50 µg/ml phleomycin. However, the sensitivity of cells is pH dependent, i.e. the higher the pH of the culture medium, the greater the sensitivity. Thus the concentration of phleomycin required for complete growth inhibition can be reduced by increasing the pH of the culture medium. In addition, the activity of phleomycin is reduced by a factor 2 to 3 in hypotonic media such as those used for protoplast regeneration. Thus, using low salt media when possible decreases the amount of phleomycin needed.

Prior to each use, bring phleomycin to room temperature and gently vortex to homogenize before use.

- **Escherichia coli**
  The cells of the common E. coli recipient strains (i.e. HB101, DH5α, MC1061) transformed by vectors carrying bleomycin resistant genes, such as Sh ble and Tn5, are resistant to phleomycin. Phleomycin-resistant transformants are selected in Low Salt LB agar medium (yeast extract 5g/l, Tryptone 10g/l, NaCl 5g/l, Agar 15 g/l, pH 7.5) supplemented with 5 µg/ml of phleomycin. Plates containing phleomycin are stable for 1 month when stored at 4 °C.

- **Yeast cells**
  Phleomycin-resistant transformants of Saccharomyces cerevisiae are selected with 10 µg/ml of phleomycin in YEPD medium. Following transformation with plasmid DNA, yeast cells are cultured in YEPD medium and incubated in a shaker for phenotypic expression of the antibiotic resistance for 6 hours to overnight. Then the culture is chilled for 1 hour on ice before plating on YEPD medium (pH 7.0) supplemented with 10 µg/ml of phleomycin.

- **Fungi**
  Phleomycin-resistant transformants are selected with 10-50 µg/ml of phleomycin in the regeneration medium, depending on the sensitivity of the host strain. Selectivity can be increased by overnight incubation at 4 °C of the selection plates prior to incubation at growth temperature.

- **Plant cells**
  Phleomycin-resistant transformants are selected with 5-25 µg/ml of phleomycin depending on the vegetal.

- **Mammalian cells**
  The working concentration of phleomycin for mammalian cell lines varies from 5 to 50 µg/ml. In a starting experiment we recommend to determine the optimal concentration of phleomycin required to kill your host cell line. The killing and the detachment of dead cells from the plate, especially at high cell density, can require a longer time compared to G418. Foci of phleomycin-resistant stable transfectants are usually individualized after 5 days to 3 weeks incubation, depending on the cell line.

TECHNICAL SUPPORT
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