

Phleomycin Powder

Selective antibiotic for the *Sh ble* gene; cell culture tested

Catalog code: ant-ph-2p, ant-ph-5p

<https://www.invivogen.com/phleomycin>

For research use only

Version 20J14-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)

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 from *Streptoalloteichus hindustanus*.

Phleomycin is a glycopeptide antibiotic of the bleomycin family, isolated from a mutant strain of *Streptomyces verticillus*. Although bleomycin antibiotics perturb plasma membranes, their activity is generally believed to be related to their ability to bind and intercalate DNA thus destroying the integrity of the double helix.

SAFETY CONSIDERATIONS

Phleomycin is a harmful compound. Refer to safety data sheet for handling instructions.

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

TECHNICAL SUPPORT

InvivoGen USA (Toll-Free): 888-457-5873

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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 hypertonic 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 5 g/L, Tryptone 10 g/L, NaCl 5 g/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 diluted 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.

RELATED PRODUCTS

Product	Description	Catalog Code
Other selection antibiotics		
Blasticidin	Selection antibiotic for the <i>bsr</i> or BSD genes	ant-bl-1
G418	Selection antibiotic for the <i>neo</i> gene	ant-gn-1
Hygromycin B Gold	Selection antibiotic for the <i>hph</i> gene	ant-hg-1
Puromycin	Selection antibiotic for the <i>pac</i> gene	ant-pr-1
Zeocin™	Selection antibiotic for the <i>Sh ble</i> gene	ant-zn-1
Plasmids encoding the <i>Sh ble</i> gene		
pMOD2-Zeo	Plasmid encoding a synthetic <i>Sh ble</i> gene	pmod2-zeo
pSELECT-zeo-LacZ	LacZ-expression plasmid selectable with Zeocin™	psetz-lacz
pSELECT-zeo-mcs	Expression plasmid selectable with Zeocin™	psetz-mcs

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