

Phleomycin

Selective antibiotic for the *Sh ble* gene

Catalog # ant-ph-1, ant-ph-5, ant-ph-5b

For research use only

Version # 15A23-MM

PRODUCT INFORMATION

Content:

Phleomycin is supplied as either 1 ml tubes or a 25 ml bottle containing a blue solution at 20 mg/ml, filtered to sterility for customer convenience, and validated for cell culture usage.

- ant-ph-1: 5 x 1 ml at 20 mg/ml (100 mg)
- ant-ph-5: 25 x 1 ml at 20 mg/ml (500 mg)
- ant-ph-5b: 1 x 25 ml at 20 mg/ml (500 mg)

Storage and stability:

- Phleomycin is shipped at room temperature. Store Phleomycin at 4 °C or at -20 °C. Phleomycin is stable for 18 months at -20 °C, 12 months at 4°C, and 1 month at room temperature. 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

Purity: >90% (HPLC)

Activity is tested using bioassays on bacteria and mammalian cell lines.

SPECIAL HANDLING

Phleomycin is a hazardous compound: avoid contact with skin, harmful if swallowed. It is readily inactivated by acidic or basic pH or by sodium hypochloride.

BACKGROUND

Phleomycin is used as a selective agent in molecular genetics experiments. Phleomycin is a glycopeptide antibiotic of the bleomycin family, isolated from a mutant strain of *Streptomyces verticillus*. It binds and intercalates DNA thus destroying the integrity of the double helix. Phleomycin 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.

Although the bleomycin antibiotics perturb plasma membranes, their activity is generally believed to be related to their ability to bind DNA by intercalation of their planar bithiazole-containing moiety. The DNA is degraded by the metal ion chelating portion of the molecule which forms an active complex with iron II and molecular oxygen.

CHEMICAL PROPERTIES

Phleomycin is a complex of structurally related antibiotics which differ by their terminal amine residues. The antibiotics are in a copper chelated form giving a blue color to the solution.

CAS number: 11006-33-0

Empirical formula: C₅₅H₈₅O₂₁N₂₀S₂Cu - HCl

Molecular weight: 1525

RESISTANCE TO PHLEOMYCIN

Phleomycin resistance is conferred by the *Sh ble* gene which encodes a small protein (14 kDa) whose structure has been characterized. The *Sh ble* protein appears to be non-toxic for a wide variety of cells in which the gene was expressed. This protein binds phleomycin with a strong affinity. The binding of phleomycin inhibits its DNA strand cleavage activity.

CONDITIONS OF SELECTION

Most cells growing aerobically are killed by phleomycin in the concentration range of 0.1 to 50 µg/ml. However, the sensitivity of cells is pH dependent, i.e. the higher the pH of culture medium, the greater the sensitivity. Thus, the concentration of phleomycin required for complete growth inhibition of given cells can be reduced by increasing the pH of the medium. In addition, the activity of phleomycin is reduced by a factor two to three 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 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.

- Yeasts

Phleomycin-resistant transformants of *Saccharomyces cerevisiae* are selected with 10 µg/ml of phleomycin in YEPD medium.

Yeast cells are transformed according to standard procedures. After DNA uptake, 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 one 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

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

InvivoGen USA (International): +1 (858) 457-5873

InvivoGen Europe: +33 (0) 5-62-71-69-39

InvivoGen Hong Kong : +852 3-622-34-80

E-mail: info@invivogen.com

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