

Phleomycin

Selective antibiotic for the *Sh ble* gene - (powder)

Catalog # ant-ph-10

For research use only

Version # 03F25-MT

PRODUCT INFORMATION

Content:

Phleomycin (#ant-ph-10) is supplied as 1 gram of a blue powder.

Storage and stability:

Phleomycin is shipped at room temperature. Upon receipt, store **Phleomycin** at 4°C for immediate use or at -20°C for long term storage.

Phleomycin is stable one week at 37°C and up to one year at 4°C.

Phleomycin is sensitive to high concentrations of acids but a short-term exposure to dilute acids can be tolerated.

Solubility:

Phleomycin is freely soluble in water, forming a light blue solution. It is slightly soluble in methanol and insoluble in more apolar solvents like acetone or chloroform.

Quality control

Retention time on reverse phase C18 column at 300 nm: 8.5 min

SPECIALHANDLING

Phleomycin is a very hygroscopic powder, keep vial tightly closed after each use to shield it from moisture.

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 DNAbly 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 n°: 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 DNAstrand 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.

- *Escherichia coli*

The cells of the common *E. coli* recipient strains (i.e. HB101, DH5 , 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

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