

# Phleomycin

Selective antibiotic for the *Sh ble* gene

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

For research use only

Version # 09K30-MM

## PRODUCT INFORMATION

### Content:

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

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

### Storage and stability:

**Phleomycin** is shipped at room temperature. Store **Phleomycin** at 4°C for several months or at -20°C for at least one year.

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

### Quality control

Purity controlled by HPLC: >90%

Activity controlled by 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 n°:** 11006-33-0

**Empirical formula:** C<sub>55</sub>H<sub>85</sub>O<sub>21</sub>N<sub>20</sub>S<sub>2</sub>Cu - 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.

### - *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

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## References

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