

Phleomycin (20 mg/mL in solution)

Product Information

Product Name	Cat#	Size
Phleomycin (20 mg/mL in solution)	60217ES20	20 mg
	60217ES60	5×20 mg

Product Description

Phleomycin is a glycopeptide antibiotic produced by a mutant strain of *Streptomyces verticillus*, belonging to the Bleomycin family. The antibacterial mechanism is that phleomycin can bind and insert into DNA, thereby damaging the integrity of its double helix structure. Saprophyacin is active against most bacteria, fungi, yeasts, plant cells, and mammalian cells, especially for cells with low sensitivity to Zeocin, such as yeast and filamentous fungi.

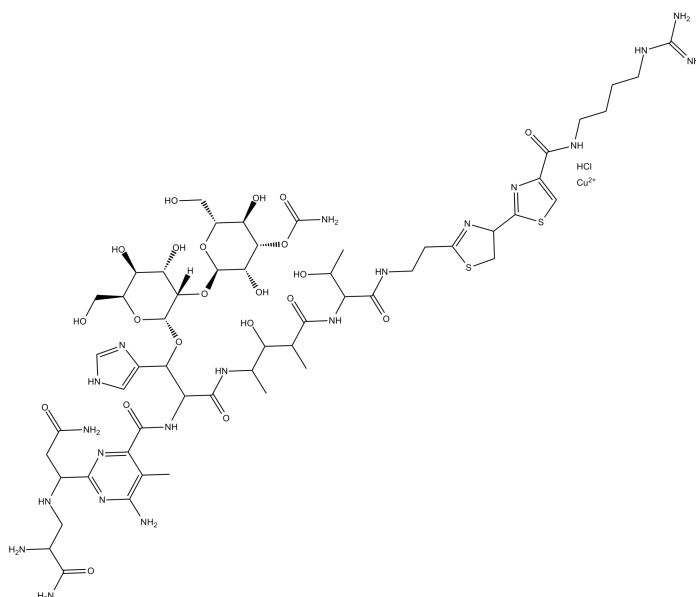
Putrescine is one of the selective antibiotics in molecular genetics research and can be used to screen transfected cells carrying *Sh ble* gene. *Sh ble* gene is derived from *Streptoalloteichus hindustanus*, which encodes 14 kDa protein that binds strongly to phleomycin and inhibits its activity of cutting DNA strands. Phleomycin is a complex of structural analogs that differ only in the terminal amine group. In addition, the solution is blue because it is a complex chelated by copper ions.

This product is provided as a sterile solution of 20 mg/mL, which can be used directly for cell culture. In the concentration range of 0.1-50 µg/mL, phleomycin can inhibit the growth of most aerobic cells.

Product Properties

Concentration	20 mg/mL in solution
CAS No.	11006-33-0
Molecular formula	C ₅₅ H ₈₅ O ₂₁ N ₂₀ S ₂ Cu•HCl
Molecular weight	1525 g/mol
Appearance	Blue liquid
Purity	≥95%
Endotoxin level	< 1.0 EU/mg

Structure



Shipping and Storage

The product is shipped with ice pack and can be stored at 4°C or -20°C. Valid for 12 months at 4°C and 18 months at -20°C. Avoid repeated freezing and thawing.

Instructions

【Note】

- 1) The sensitivity of cells to phleomycin is affected by pH value. For example, the higher the pH value, the higher the sensitivity. In addition, for high osmotic pressure medium such as medium for protoplast regeneration, the activity of phleomycin will be significantly reduced by 2-3 times. It is therefore also possible to reduce the amount of phleomycin used by using a low-salt medium.
- 2) Before use, the phleomycin stored at low temperature should be brought back to room temperature, and then fully mixed at low speed.

1. *E. coli*

Plasmids carrying *bleomycin* resistance genes such as *Sh ble*, *Tn5*, once transformed into *Escherichia coli* bacteria such as HB101, DH5a and MC1061, endow them with phleomycin resistance.

Phleomycin-resistant transformants can be grown on low-salt LB agar medium supplemented with 5 µg/mL Phleomycin (Yeast extract 5 g/L, Tryptone 10 g/L, NaCl 5 g/L, Agar 15 g/L, pH 7.5) for screening. Plates supplemented with phleomycin are stable for 1 month at 4°C.

2. Yeast

Phleomycin-resistant transformants of *S. cerevisiae* can be selected in YEPD medium supplemented with 10 µg/mL Phleomycin.

Procedure: Yeast cell transformation is carried out according to the conventional method. Once the DNA has entered the cells, the cells are diluted with YEPD medium and cultured on a shaker for 6 h or overnight to express the resistance traits. The cells were then incubated on ice for 1 h and plated on YEPD (pH 7.0) solid medium supplemented with 10 µg/mL Phleomycin.

3. fungus

Depending on the sensitivity of transformants to Phleomycin, selection is performed in regeneration medium supplemented with 10-50 µg/mL phleomycin. Sensitivity to antibiotics can be increased by incubating cells overnight at 4°C and then at the growth temperature.

4. Plant cell

Select 5-25 µg/mL Phleomycin for transformant screening depending on plant type

5. Mammalian cell

Phleomycin: 5 to 50 µg/mL. It is recommended to determine the optimal concentration by establishing a kill curve. Compared to G418, it may take longer to kill cells or to shed them from the plate, especially if the cells are at high density.

Depending on the cell line, it usually takes 5-21 days to select phleomycin-resistant stably transfected cells.

Cautions

1. Saprophycin is sensitive to concentrated acids but can be exposed to dilute acids for a short time. Warm the product to room temperature and mix gently before use.
2. Saprophycin is poisonous, avoid direct contact with skin, do not swallow. In acidic or alkaline pH, or sodium hypophosphate, it will immediately lose effect.
3. For your safety and health, please wear lab coats and disposable gloves for operation.
4. For research use only!