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V 1.0.0

Bleomycin hydrochloride

Product Description:

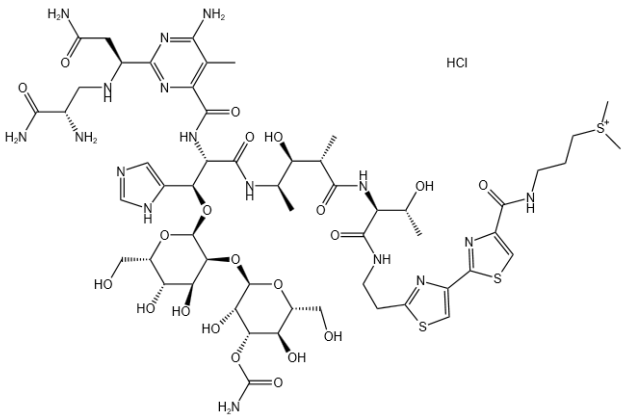
Bleomycin hydrochloride is a member of the bleomycin/phleomycin antibiotic family. It is a water-soluble, copper-chelating glycopeptide antibiotic isolated from Streptomyces verticillus; the presence of the copper ion gives the solution a blue color. This copper-chelated form is inactive: after the antibiotic enters the cell, Cu^{2+} is reduced to Cu^{+} and is removed by thiol compounds in the cell. Only then is bleomycin hydrochloride activated; it binds to and cleaves DNA, thereby causing cell death. The protein encoded by the bleomycin hydrochloride resistance gene (Sh ble) binds to bleomycin hydrochloride and inhibits its DNA cleavage. Bleomycin hydrochloride has a very broad activity spectrum, with strong antimicrobial activity against bacteria, fungi (including yeasts), plants, and mammalian cells. It is therefore widely used as a highly effective tool antibiotic for selecting cell lines that carry the Sh ble resistance gene and stably express the 13.665 kDa bleomycin hydrochloride resistance protein.

This product is supplied as a sterile solution in high-purity water at a concentration of 100 mg/mL, cell culture grade.

Chemical Properties:

CAS: 67763-87-5

Molecular Formula: $C_{55}H_{84}ClN_{17}O_{21}S_3$
Molecular Weight: 1451
Purity: >95% by HPLC
Structural Formula:



Product Information:

Product Number	Product Name	Specifications
GM-040407-100MG	Bleomycin hydrochloride	100 mg
GM-040407-1G	Bleomycin hydrochloride	100 mg×10

Storage Conditions:

Shipped with ice packs. Store the product at -20° C protected from light. Avoid repeated freeze-thaw cycles; the shelf life is 1 year.

Working Concentrations:

1. **E. coli:** 25-50 μg/mL; select with low-salt LB medium (NaCl concentration must not exceed 5 g/L).
2. **Mammalian cells:** 50-1000 μg/mL; choose a suitable medium according to the cell type.

Protocol (Mammalian Cell Selection):

Precautions

1) Bleomycin hydrochloride is used to select stably transfected mammalian cells at concentrations of 50-1000 μg/mL, with an average working concentration range of 250-400 μg/mL. The main factors affecting the selection concentration include ionic strength, cell type, cell density, and growth rate. The following steps are provided for reference only; please adjust them appropriately for your own experimental system.
2) The killing mechanism of bleomycin hydrochloride differs from that of neomycin and hygromycin: cells do not clump together or detach from the plate surface. Upon exposure to bleomycin hydrochloride, sensitive cells undergo the following morphological changes: a) a marked increase in cell volume (similar to the swelling effect caused by cytomegalovirus infection of permissive cells); b) abnormal cell shapes, including the appearance of long appendages; c) large cytoplasmic vacuoles (derived from rupture of the endoplasmic reticulum, Golgi apparatus, or structural proteins); d) rupture of the plasma membrane and nuclear envelope, leaving many holes in the membranes. These sensitive cells eventually break down completely and remain only as cell debris.
3) Bleomycin hydrochloride-resistant cells continue to grow normally and form colonies distinct from those of sensitive cells. Morphologically, they are indistinguishable from normal cells that have never been exposed to bleomycin hydrochloride.

1. Establishing a Kill Curve

1) Re-plate or split confluent cells to a density of approximately 25%. Prepare 8 plates per group and culture for 24 h.
2) Remove the old medium and add selection medium containing different concentrations of bleomycin hydrochloride. The concentration may be set at 0, 50, 100, 200, 400, 600, 800 and 1000 μg/mL, each in triplicate; different concentration gradients may also be set according to your own experimental system.
3) Replace with fresh selection medium every 3-4 days and monitor the proportion of surviving cells. The optimal working concentration is the one that kills the majority of cells within an appropriate time (1-2 weeks).

Note: If it is difficult to distinguish viable cells under the microscope, trypan blue staining may also be used to count viable cells in order to determine the optimal concentration of bleomycin hydrochloride.

2. Selection of Stably Transfected Cells

1) Transfect the cells and culture them in 100 mm dishes. Use non-transfected cells as a negative control.
2) After transfection, wash the cells once with pre-warmed 1× PBS and add fresh medium.
3) 48-72 h after transfection, select the cells with fresh medium containing the optimal concentration of bleomycin hydrochloride (as determined by the kill curve). For easier identification and isolation of colonies, it is recommended to serially dilute the cells into a range of concentrations.

Note: If the cells to be selected show markedly stronger resistance to bleomycin hydrochloride than most cells, or if rapid cell division makes the selection effect of bleomycin hydrochloride at low concentrations inconspicuous, overcome such resistance as follows: a) split the cells directly into selection medium containing bleomycin hydrochloride; b) incubate at 37°C for 2-3 h until the cells attach; c) remove the cells from the incubator and hold them at 4°C for 2 h, making sure the medium contains HEPES [the purpose of this step is to halt cell division within a short time, allowing bleomycin hydrochloride resistance to take effect and kill the cells]; d) return the cells to the 37°C incubator for culture.

4) Add fresh selection medium every 3-4 days until colonies appear.
5) Pick and transfer colonies into 96- or 48-well plates. Before expanding to larger plates or dishes, make sure the cells have reached near 100% confluence.

3. Maintenance of Stably Transfected Cells

Stably transfected cells may be maintained in any of the following ways: 1) maintain the culture in selection medium containing the same dose of bleomycin hydrochloride; 2) reduce the dose of bleomycin hydrochloride to half of the original; 3) use a dose of bleomycin hydrochloride just sufficient to prevent the growth of sensitive cells without being lethal [refer to the kill curve].

Precautions:

1. This product is somewhat light-sensitive. Carry out handling and culture in the dark whenever possible, and store the product protected from light.
2. This product is toxic. For your safety and health, please wear a lab coat and disposable gloves when handling.
3. This product is for research use only. Not for use in humans.