

Zeocin™

Phleomycin, Ceozin

Isolated from *Streptomyces verticillus*

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Product	Cat#	Package size
Zeocin™ powder	M3446.0250	250mg
Zeocin™ powder	M3446.0001	1g
Zeocin™ powder	M3446.0005	5g

Product description

Zeocin™ is used as a selective agent in molecular genetics experiments. Zeocin™ is the commercial name of a special formulation of Phleomycin D1, a glycopeptide antibiotic of the bleomycin family, isolated from a mutant strain of *Streptomyces verticillus*. It binds and intercalates DNA thus destroying the double helix structure. Zeocin™ (Phleomycin) exhibits activity against most bacteria, filamentous fungi, yeast, plant and animal cells. Because of its broad spectrum of toxicity, Zeocin™ is particularly useful for identification and selection of a variety of cell types harbouring vectors carrying Zeocin™ resistance genes. If Zeocin™ shows no effect it is recommended to use Phleomycin, especially for cells poorly sensitive to Zeocin™ i.e. filamentous fungi and yeast.

Although the bleomycin (like Phleomycin, Zeocin™) antibiotics perturb plasma membrane, 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 proportion of the molecule which forms an active complex with iron II and molecular oxygen.

Most cells growing aerobically are killed by Phleomycin at concentrations of 0.1 µg/mL to 50 µg/mL and 0.5 µg/mL to 1000 µg/mL of Zeocin™. 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 Zeocin™/phleomycin required for complete growth inhibition of given cells can be reduced by increasing the pH of the medium. In addition, the activity of Zeocin™/phleomycin is reduced by a factor 2 to 3 in hypertonic media such as those used for protoplast regeneration. Thus, using low salt media when possible decreases the amount of Zeocin™/phleomycin needed.

Physical and chemical Properties

Category: Peptide antibiotic, DNA strand scission agent.

Binding mode: Intercalation

Appearance

Zeocin™/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 colour to the solution and to the salt (powder). The amorphous powder is very soluble in water resulting in a blue solution. It is slightly soluble in alcohol and insoluble in more apolar solvents like acetone or chloroform.

Zeocin™/Phleomycin is a labile compound which undergoes irreversible denaturation at high or low pH or in presence of a weak oxidant. Even sensitive to high concentrations of acids, a short-term exposure to dilute acids can be tolerated.

Zeocin™/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.

Resistance to Phleomycin

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

As there is no cross resistance with other currently used drug resistance markers, Zeocin™/Phleomycin can be used to select cells resistant to other selective agents (i.e. G418, hygromycin B, blasticidin S or puromycin).

Storage, stability and purity:

- Zeocin™/Phleomycin is shipped at RT, but has to be stored at +4 °C (short term), or -20 °C (long term).
- At +4 °C Zeocin™/Phleomycin might be stable for up to 1 year.
- Zeocin™/Phleomycin is **highly hygroscopic**. Keep tubes tightly closed after each use.
- Zeocin™/Phleomycin is sensitive to high concentrations of acids but a short-term exposure to diluted acids is tolerable.
- Zeocin™/Phleomycin elutes from a C18 reversed phase column at 8.5 minutes as a single peak.

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Conditions of Selection

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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 Zeocin™/phleomycin required for complete growth inhibition of given cells can be reduced by increasing the pH of the medium. In addition, the activity of Zeocin™/phleomycin is reduced by a factor 2 to 3 in hypertonic media such as those used for protoplast regeneration. Thus, using low salt media when possible decreases the amount of Zeocin™/phleomycin needed.

- Escherichia coli

The cells of the common *E.coli* recipient strains (i.e. HB101, DH5a, MC1061) transformed by vectors carrying bleomycin resistant genes, such as *Sh ble* and Tn5, are resistant to Zeocin™/phleomycin.

NOTE: Do not use an *E.coli* recipient strain that contains the Tn5 transposable element (i.e. MC1066). Tn5 encodes a bleomycin resistance gene that will confer resistance to Zeocin™/phleomycin.

Zeocin™/Phleomycin-resistant transformations are selected in Low Salt LB agar medium (yeast extract 5g/L, Tryptone 10g/L, NaCl 5g/L, Agar 15g/L, pH7.5) supplemented with 5µg/mL of phleomycin or 25µg/mL of Zeocin™. Plates containing Zeocin™/phleomycin are stable for 1 month stored at +4°C.

- Yeasts (Not for Zeocin™ - only Phleomycin)

Phleomycin-resistant transformants of *Saccharomyces cerevisiae* are selected with 10µg/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 (pH7.0) supplemented with 10µg/mL of phleomycin.

- Fungi (Not for Zeocin™ - only Phleomycin)

Phleomycin-resistant transformants are selected with 10-50µg/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 (Not for Zeocin™ - only Phleomycin)

Phleomycin-resistant transformants were selected with 5-25µg/mL of phleomycin depending on the vegetal.

- Mammalian cells

The working concentration of Zeocin™ for mammalian cell lines varies from 25µg/mL to 1000µg/mL. The working concentration of Phleomycin for mammalian cell lines varies from 5µg/mL to 50µg/mL. In a starting experiment we recommend to determine the optimal concentration of Zeocin™/Phleomycin required to kill your host cell lines. 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 Zeocin™/phleomycin-resistant stable transfectants are usually individualised after 5 days to 3 weeks incubation, depending on the cell line. Suggested concentrations of Zeocin™ and Phleomycin for selection in mammalian cells are listed below.

Table 1: Suggested concentration of Zeocin™ for selection

Cell line	Species	Tissue	Culture medium	Zeocin™ µg/mL*
293	Human	Kidney	DMEM	100
HeLa	Human	Uterus	DMEM	100
MCF-7	Human	Breast adenocarcinoma	DMEM	100
WiDr	Human	Colorectal adenocarcinoma	DMEM	50-100
B16	Mouse	Melanoma	RPMI	20-50
C2C12	Mouse	Myoblast	DMEM	250-500
CHO	Hamster	Ovary	DMEM	100-250
PC1.0	Hamster	Pancreatic adenocarcinoma	RPMI	200-400
C6	Rat	Glioma	DMEM	100-200
COS	Monkey	Kidney	DMEM	250

*Source: InvivoGen

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Methodology

Preparation of Zeocin™/Phleomycin Solutions

1. Re-suspend Zeocin™/Phleomycin in water or HEPES buffer (pH7.25) at a concentration of 100mg/mL (Zeocin™) or 20mg/mL (Phleomycin).
2. Sterile filter solution using a 0.22µm sterile filter.
3. Store solution at +4°C for immediate use or at -20°C for long term storage.

Selection procedure for mammalian cells

Zeocin™ is normally used at a concentration of 100µg/mL, Phleomycin at a concentration of 20µg/mL a 1000-fold dilution from stock solution. After transformation with a plasmid containing the Sh ble gene, cells are incubated in their regular growth medium containing Zeocin™ or Phleomycin to select for stable transfectants.

1: 48 hours post-transfection, pass cells (direct or diluted) in fresh medium containing Zeocin™ at the appropriate concentration.

NOTE: Antibiotics work best when cells are actively dividing. If the cells become too dense, the antibiotic efficiency will decrease. It is best to split cells such that they are not more than 25% confluent.

2: Remove and replace antibiotic containing medium every 3-4 days.

3: Evaluate cells for the formation of foci after 7 days of selection. Foci may require an additional week or more to develop depending on the host cell line and transfection/selection efficiency.

4: Transfer and pool 5-10 resistant clones to a 35mm cell culture plate and maintain on selection medium for an additional 7 days. This pooled culture will be expanded for subsequent cytotoxicity assays.

References

1. Drocourt, D., Calmels, T., Reynes, J.P., Baron, M. and G. Tiraby, 1990.
Cassettes of the *Streptoalloteichus hindustanus* ble gene for transformation of lower and higher eukaryotes to phleomycin resistance. Nucl. Acids. Res. 18: 4009.
2. Gagnon, A., Durandt, H. and G. Tiraby, 1988.
Bleomycin resistance conferred by a drug-binding protein. FEBS Letters 230: 171-175.
3. Dumas, Ph., Bergdoll, M., Cagnon C. and J.M. Masson, 1994.
The three-dimensional structure of a bleomycin resistance protein. EMBO J. 242 (5) 595-601.
4. Li, F., Wilkins, P.P., Crawley, S., Weinstein, J. Cummings, R.D. and R.P. McEver, 1996.
Post-translational modifications of recombinant P-selection glycoprotein ligand-1 required for binding to P- and E-selection. J. Biol. Chem. 271: 3255-3264.
5. Feng, Y., Broder, C.C., Kennedy, P.E. and E.A. Berger, 1996.
HIV-1 entry cofactor : functional c-DNA cloning of a seven-transmembrane, G-protein-coupled receptor. Science, 272: 872-877.
6. Apt, K.E., Kroth-Pancic, P.G. and A.R. Grossman, 1996.
Stable nuclear transformation of the diatom *Phaedactylum tricornutum*. Mol. Gen. Genet. 252: 572-579.
7. Bouayadi, K., Hoffmann, J.S., Fons, P., Tiraby, M., Reynes, J.P. and C. Cazaux, 1997.
Overexpression of DNA polymerase beta sensitizes mammalian cells to 2',3'-deoxycytidine and 3'-azido-3'-deoxythymidine. Cancer Res. 57: 110-116.
8. Pfeifer, R.A., Hegedus, D.D., Grigliatti, T.A. and D.A. Theilman, 1997.
Baculovirus immediate-early promoter-mediated expression of the Zeocin™ resistance gene for use as a dominant selectable marker in Dipteran and Lepidopteran insect cell lines. Gene. 188: 183-190
9. Bagnis, C., Gravis, G., Imbert, A.M., Herrera, D., Allario, T., Galindo, R.k Lopez, M., Pavon, C., Semprere, C., and P. Mannoni, 1994.
Retroviral transfer of the nslacZ gene into human CD34+ cell populations and into TF-1 cells. Future prospects in gene therapy. Hum. Gene Ther. 5(11): 1325-1333.