

Zeocin™ Powder

Selective antibiotic for the *Sh ble* gene

Catalog # ant-zn-1p, ant-zn-5p

For research use only

Version #10D26-SA

PRODUCT INFORMATION

Contents:

Zeocin™ is supplied as a blue powder.

- **ant-zn-1p:** 1 x 1 g tube.
- **ant-zn-5p:** 1 x 5 g bottle.

Note: As a powder, Zeocin™ is very hygroscopic. Keep tubes tightly closed after each use.

Storage and stability:

Zeocin™ is shipped at room temperature. **Zeocin™** powder is stable for at least one year at 4°C. Since **Zeocin™** is so hygroscopic it should be shielded from moisture.

Zeocin™ is sensitive to high concentrations of acids and bases but a short-term exposure to dilute acids can be tolerated.

Quality control

Purity controlled by HPLC and microbiological assays: >90%

SPECIAL HANDLING

Zeocin™ is a hazardous compound: avoid contact with skin, do not inhale powder particles, harmful if swallowed. It is readily inactivated by acidic or basic pH or by sodium hypochloride.

BACKGROUND

Zeocin™ is used as a selective agent in molecular genetics experiments. **Zeocin™** is the commercial name of a special formulation containing Phleomycin D1, a copper-chelated glycopeptide antibiotic isolated from culture broth of a *Streptomyces verticillus* mutant. This antibiotic of the bleomycin family exhibits activity against bacteria, eukaryotic microorganisms, 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 harboring vectors carrying **Zeocin™** resistance genes.

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. Expression of a bacterial **Zeocin™** resistance protein, the product of the *Sh ble* gene¹, allows selection of drug-resistant cells after gene transfer. Since **Zeocin™** is active in both bacteria and mammalian cell lines, vectors need only one drug resistance marker for selection.

CHEMICAL PROPERTIES

Zeocin™ 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. **Zeocin™** is a labile compound which undergoes irreversible denaturation at high and low pH or in presence of a weak oxidant.

Zeocin™ is freely soluble in water (>500 mg/ml) forming a blue solution. It is slightly soluble in methanol and insoluble in more apolar solvents like acetone or chloroform

CAS n°: 11006-33-0

RESISTANCE TO ZEOCIN™

The **Zeocin™** resistance gene (*Sh ble* gene) encodes a small protein (14 kDa) whose structure has been characterized^{2,3}. The *Sh ble* protein appears to be non-toxic for a wide variety of cells in which the gene was expressed. This protein binds **Zeocin™** with a strong affinity. The binding of **Zeocin™** inhibits its DNA strand cleavage activity. As there is no cross resistance with other currently used drug resistance markers, **Zeocin™** can be used to select cells resistant to other selective agents (i.e. G418, hygromycin B, blasticidin S or puromycin).

CONDITIONS OF SELECTION

Most cells growing aerobically are killed by **Zeocin™** in the concentration range of 0.5 to 1000 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 **Zeocin™** required for complete growth inhibition of given cells can be reduced by increasing the pH of the medium. In addition, the activity of **Zeocin™** 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 **Zeocin™** needed.

- *Escherichia coli*

The *Sh ble* gene and the hybrid genes in vectors provided by InvivoGen are driven by synthetic *E. coli* promoters (i.e EM7). The cells of the common *E. coli* recipient strains (i.e HB101, DH5α, MC1061) transformed by these vectors are resistant to **Zeocin™**.

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™.

Zeocin-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 25µg/ml of **Zeocin™**. Plates containing **Zeocin™** are stable for 1 month when stored at 4°C. For optimum results the use of InvivoGen's FastMedia™ Zeo is recommended.

- Mammalian cells

The working concentration of **Zeocin™** for mammalian cell lines varies from 25 to 1000 µg/ml. In a starting experiment we recommend to determine the optimal concentration of **Zeocin™** 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 **Zeocin-resistant stable transfectants** are usually individualized after 5 days to 3 weeks incubation, depending on the cell line. Suggested concentrations of **Zeocin™** for selection in mammalian cells are listed below.

TECHNICAL SUPPORT

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Cell line	Species	Tissue	Culture medium	Zeocin™ µg/ml	Ref. / Source
293	Human	Kidney	DMEM	100	in-house testing
HeLa	Human	Uterus	DMEM	100	in-house testing
MCF-7	Human	Breast adeno- carcinoma	DMEM	100	in-house testing
WiDr	Human	Colorectal adenocarcinoma	DMEM	50-100	in-house testing
B16	Mouse	Melanoma	RPMI	20-50	⁴⁻⁷
C2C12	Mouse	Myoblast	DMEM	250-500	in-house testing
CHO	Hamster	Ovary	DMEM	100-250	⁴⁻⁵
PC1.0	Hamster	Pancreatic adenocarcinoma	RPMI	200-400	in-house testing
C6	Rat	Glioma	DMEM	100-200	in-house testing
COS	Monkey	Kidney	DMEM	250	⁵

METHOD

Preparation of Zeocin solution

- 1- Resuspend Zeocin™ in water or HEPES buffer (pH 7.25) at a concentration of 100 mg/ml.
- 2- Sterile filter the solution using a 0.22µm sterile filter.
- 3- Store at 4°C for immediate use or -20°C for long term storage

Selection procedure for mammalian cells

Zeocin™ is normally used at a concentration of 100µg/ml, a 1000-fold dilution from the stock solution. After transformation with a plasmid containing the *Sh ble* gene, cells are incubated in their regular growth medium containing **Zeocin™** 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., T. CALMELS, J.P. REYNES, M. BARON 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- GATIGNOL, A., DURAND, H. and G. TIRABY. 1988. Bleomycin resistance conferred by a drug-binding protein. FEBS Letters. 230 : 171-175
- 3- DUMAS, Ph., M. BERGDOLL, C. CAGNON 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 Phaeodactylum 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, T.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., LOPEZ, M., PAVON, C., SEMPERE, C., and P. MANNONI. 1994. Retroviral transfer of the nlsLacZ gene into human CD34+ cell populations and into TF-1 cells: future prospects in gene therapy. Hum. Gene Ther. 5(11):1325-1333

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