

Zeocin™

Selective antibiotic for the *Sh ble* gene

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

For research use only

Version #10D26-SA

PRODUCT INFORMATION

Contents:

Zeocin™ is supplied as either 2 ml tubes or a 50 ml bottle of a 100 mg/ml solution (100% active product) in HEPES buffer, pH 7.25, filtered to sterility for customer convenience, and validated for cell-culture usage.

- **ant-zn-1:** 5 x 2 ml at 100 mg/ml (1 g)
- **ant-zn-5:** 25 x 2 ml at 100 mg/ml (5 g)
- **ant-zn-5b:** 1 x 50 ml at 100 mg/ml (5 g)

Storage and stability:

Zeocin™ is shipped at room temperature. **Zeocin™** solution is stable for at least one year at -20°C, and may be kept at 4°C several months.

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, 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.

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 adenocarcinoma	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 (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

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