Zeocin™

Selective antibiotic for the *Sh ble* gene

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

For research use only

Version # 15A27-MM

PRODUCT INFORMATION

Contents:

Zeocin™ is supplied as either 1 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:** 10 x 1 ml at 100 mg/ml (1 g)
- **ant-zn-5:** 50 x 1 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. Upon receipt it should be stored at 4 °C or -20 °C. Zeocin™ solution is stable for 18 months at -20 °C, 12 months at 4 °C, and 1 month at room temperature. Avoid repeated freeze-thaw cycles.

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

Quality control:

Purity: >90% (HPLC)

Activity is tested using microbiological assays

BACKGROUND

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* gene[1], 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.

1. Drocourt D. *et al.*, 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. *et al.*, 1988.** Bleomycin resistance conferred by a drug-binding protein. FEBS Letters. 230: 171-5. **3. Dumas P. *et al.*, 1994.** The three dimensional structure of a bleomycin resistance protein. Embo J. 242 (5) 595-601.

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 number: 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 of two to three in hypertonic media, such as those used for protoplast regeneration. Thus, using low salt medium 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 5 g/l, Tryptone 10 g/l, NaCl 5 g/l, Agar 15 g/l, pH 7.5) supplemented with 25 µg/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 50 to 400 µg/ml, in a few cases can be as low as 20 µg/ml or as high as 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 overleaf.

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WORKING CONCENTRATIONS

Zeocin™ is normally used at a concentration of 100 µg/ml, a 1000-fold dilution from the stock solution. However, the optimal concentration needs to be determined for your cells. Suggested concentrations of Zeocin™ for selection in some examples of mammalian cells are listed below.

Cell line	Species	Tissue	Medium	Zeocin™ µg/ml	Citation
B16	Mouse	Melanoma	RPMI	20-250	1, 2, 3
CHO	Hamster	Ovary	DMEM	100-500	1, 4, 5
COS	Monkey	Kidney	DMEM	100-400	6, 7
HEK293	Human	Kidney	DMEM	100-400	8, 9
HeLa	Human	Uterus	DMEM	50-100	10, 11
J558L	Mouse	Melanoma	RPMI	400	12
MCF-7	Human	Breast adenocarcinoma	DMEM	100-400	13, 14
MEFs	Mouse	Embryonic fibroblasts	DMEM	200-400	15, 16
THP-1	Human	Leukemic monocytes	RMPI	200	17

GENERAL GUIDELINES

Successful transfection is influenced by many factors. The health and viability of the cell line, the quality of the nucleic acid used, the transfection reagent, the duration of transfection, and the presence or absence of serum can all play a part. Several methods for transfection of nucleic acids are available, including treatment with DEAE-dextran, calcium phosphate, viruses or cationic lipids, and electroporation. For stable transfection using cationic lipids, we recommend LyoVec™, a lyophilized transfection reagent with low cytotoxicity.

METHOD (Selection procedure for mammalian cells)

Forty-eight hours after transfection with a plasmid containing the *Sh ble* gene, cells are incubated in their regular growth medium containing Zeocin™ to select for stable transfectants. In order to isolate monoclones, the limiting dilution method is used. With this method, cells are seeded at very low densities (with an average of 0.3 cells per well). A protocol for clone selection and expansion is provided below.

1. The day before transfection, determine the number of cells needed for transfection. Seed the cells at the optimal density and leave overnight.
2. On the day of transfection, determine the viability and the amount of cell clumping from a small aliquot of cells using the trypan blue dye exclusion method.Viability of cells must be over 90%.
Note: For best results, make sure to have a single cell suspension.
3. Prepare plasmid DNA transfection reagent complex.
4. Seed cells in 1 ml of culture medium per well of a 12-well plate.
Note: With LyoVec™, the transfection can be performed immediately after cell seeding.
5. Add 50 µl of the DNA-transfection reagent complex to each well and mix gently by rocking the plate back and forth.
Note: Do not add selection antibiotic immediately after transfection, as this will drastically increase mortality. Allow at least 48 hours before adding the selection antibiotic.
6. Prior to each use, bring Zeocin™ to room temperature and vortex to homogenize.
7. Forty-eight hours post-transfection, pass cells (direct or diluted) in fresh medium containing Zeocin™ at the appropriate concentration. Ensure that cells are in the exponential growth phase.
Note: Antibiotics work best when cells are actively dividing. If the cells become too dense, the antibiotic efficiency will decrease.
8. Replace the medium with fresh antibiotic-containing medium every 3 days.
9. Once a population of resistant cells has been obtained, clone these cells at a density of 0.3 cells/well in a 96-well plate.
Notes:
- The ratio of 0.3 cells/well provides a very low chance of having two cells in the same well.
- To obtain a seeding density of 0.3 cells/well, add 200 ml of a cell suspension of ~1.5 cell/ml in antibiotic-containing medium.
10. After 4 days, assess the number of wells with colonies.
Note: To obtain clonal colonies, as a general rule, the number of positive wells should not exceed 30 per plate.
11. After these wells have been identified, verify cell growth every week. It normally takes 3 weeks to obtain sufficient cells, however, this depends on the growth rate of your cells.
12. Expand the selected single-colony wells.
13. Verify gene expression using the appropriate assays.
14. Upon establishing your target monoclonal stable cell line, a lower amount of antibiotic may be used for maintenance.

Citations (articles featuring Zeocin™)

1. Bouayadi K. *et al.*, 1997. Overexpression of DNA polymerase beta sensitizes mammalian cells to 2',3' deoxycytidine and 3'-azido-3'-deoxythymidine. Cancer Res. 57: 110-116. **2. Hirose Y. *et al.*, 2012.** Inhibition of Stabilin-2 elevates circulating hyaluronic acid levels and prevents tumor metastasis. PNAS, 109: 4263 - 4268. **3. Fan H. *et al.*, 2012.** Intracerebral CpG immunotherapy with carbon nanotubes abrogates growth of subcutaneous melanomas in mice. Clin Cancer Res.18(20):5628-38. **4. Li F. *et al.*, 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. Ogura T. *et al.*, 2004.** Resistance of B16 melanoma cells to CD47-induced negative regulation of motility as a result of aberrant N-glycosylation of SHPS-1. J Biol Chem. 279(14):13711-20. **6. Saxena A. *et al.*, 2002.** H2, the minor subunit of the human asialoglycoprotein receptor, trafficks intracellularly and forms homo-oligomers, but does not bind asialo-orosomucoid. J Biol Chem. 277(38):35297-304. **7. Kanamori A. *et al.*, 2002.** Distinct sulfation requirements of selectins disclosed using cells that support rolling mediated by all three selectins under shear flow. L-selectin prefers carbohydrate 6-sulfation totyrosine sulfation, whereas p-selectin does not. J Biol Chem. 277(36):32578-86. **8. Ahmed *et al.*, 2013.** TRIF-mediated TLR3 and TLR4 signaling is negatively regulated by ADAM15. J Immunol. 190(5):2217-28. **9. Büllesbach EE. & Schwabe C., 2006.** The mode of interaction of the relaxin-like factor (RLF) with the leucine-rich repeat G protein-activated receptor 8. J Biol Chem. 281(36):26136-43. **10. Mesnil M. *et al.*, 1996.** Bystander killing of cancer cells by herpes simplex virus thymidine kinase gene is mediated by connexins. PNAS 93(5):1831-5. **11. Maszczak-Seneczko D. *et al.*, 2013.** UDP-N-acetylglucosamine transporter (SLC35A3) regulates biosynthesis of highly branched N-glycans and keratan sulfate. J Biol Chem. 288(30):21850-60. **12. Cedeno-Laurent F. *et al.*, 2010.** Development of a nascent galectin-1 chimeric molecule for studying the role of leukocyte galectin-1 ligands and immune disease modulation. J Immunol. 185(8):4659-72. **13. Kim HS. *et al.*, 2004.** Insulin-like growth factor-binding protein 3 induces caspase-dependent apoptosis through a death receptor-mediated pathway in MCF-7 human breast cancer cells. Cancer Res. 64(6):2229-37. **14. List HJ. *et al.*, 2001.** Ribozyme targeting demonstrates that the nuclear receptor coactivator AIB1 is a rate-limiting factor for estrogen-dependent growth of human MCF-7 breast cancer cells. J Biol Chem. 276(26):23763-8. **15. Waak J. *et al.*, 2009.** Oxidizable residues mediating protein stability and cytoprotective interaction of DJ-1 with apoptosis signal-regulating kinase 1. J Biol Chem. 284(21):14245-57. **16. MacDonald M. *et al.*, 2007.** The zinc finger antiviral protein acts synergistically with an interferon-induced factor for maximal activity against alphaviruses. J Virol. 81(24):13509-18. **17. Maue A. *et al.*, 2013.**The polysaccharide capsule of Campylobacter jejuni modulates the host immune response. Infect Immun. 81(3):665-72.

RELATED PRODUCTS

Product	Catalog Code
LyoVec™	lyec-12
Other Selective Antibiotics:	
Blasticidin	ant-bl-1
G418	ant-gn-1
Hygromycin B Gold	ant-hg-1
Puromycin	ant-pr-1

TECHNICAL SUPPORT
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