PRODUCT INFORMATION

CL990-cin

Product information for Z706211:

Description

CL990-cin is a selection antibiotic that acts on both eukaryotic and prokaryotic cells. Resistance to CL990-cin is conferred by the *Sh ble* gene from *Streptoalloteichus hindustanus*1-3. CL990-cin is the commercial name for a special formulation containing Phleomycin, a copper-chelated glycopeptide antibiotic isolated from a mutant strain of *Streptomyces verticillus*. This antibiotic of the bleomycin family exhibits activity against bacteria, eukaryotic microorganisms, plant and animal cells. Although bleomycin antibiotics perturb plasma membranes, their activity is generally believed to be related to their ability to bind and intercalate DNA thus destroying the integrity of the double helix.

Chemical Properties

CL990-cin is a mixture of structurally related antibiotics which differ by their terminal amine residues. The antibiotics are in a copper chelated form giving the solution a blue color. CL990-cin is a labile compound which undergoes irreversible denaturation at high and low pH or in presence of a weak oxidant. CL990-cin is freely soluble in water (>500 mg/ml) forming a blue solution.

Preparation

1. Resuspend CL990-cin in HEPES buffer (5 g/l, pH 7.2+/- 0.1) at a concentration of 100 mg/ml.
2. Sterile filter the solution using a 0.22µm sterile filter.
3. Store at 4°C for 12 months or -20 °C for 18 months.

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.

Condition of Selection

Most cells growing aerobically are killed by CL990-cin 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 CL990-cin required for complete growth inhibition of given cells can be reduced by increasing the pH of the medium. In addition, the activity of CL990-cin is reduced by a factor of two to three in hypertonic media, such as those used for protoplast regeneration. Thus, using low salt media when possible decreases the amount of CL990-cin 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, DH5a,

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MC1061) transformed by these vectors are resistant to CL990-cin.
***Note**: Do not use an E. coli recipient strain that contains the Tn5 transposable element (i.e. MC1066). Tn5 encodes a bleomycinresistance gene that will confer resistance to* CL990-cin*.*

CL990-cin -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 25 µg/ml of CL990-cin. Plates containing CL990-cin are stable for 1 month when stored at 4°C.

- Mammalian cells
The working concentration of CL990-cin 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 CL990-cin 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 CL990-cin -resistant stable transfectants are usually individualized after 5 days to 3 weeks incubation, depending on the cell line. Suggested concentrations of CL990-cin for selection in mammalian cells are listed on the next page.

Working Concentration
CL990-cin 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 CL990-cin for selection in some examples of mammalian cells are listed below.

Cell line	**Medium**	CL990**-cin conc**	**References**
B16 (Mouse melanocytes)	RPMI	20-250 µg/ml	4-6
CHO (Chinese hamster ovarian cells)	DMEM	100-500 µg/ml	4, 7, 8
COS (Monkey kidney cells)	DMEM	100-400 µg/ml	9, 10
HEK293 (Human embryonic kidney cells)	DMEM	100-400 µg/ml	11, 12
HeLa (Human uterine cells)	DMEM	50-100 µg/ml	13, 14
J558L (Mouse melanocytes)	RPMI	400 µg/ml	15
MCF-7 (Human breast adenocarcinoma cells)	DMEM	100-400 µg/ml	16, 17
MEFs (Mouse embryonic fibroblasts)	DMEM	200-400 µg/ml	18, 19
THP-1 (Human monocytes)	RMPI	200 µg/ml	20

Storage/Stability
- CL990-cin is shipped at room temperature. Upon receipt it should be stored at 4°C. The expiry date is specified on the product label.
- Store CL990-cin solutions at 4°C or -20°C. Do not use CL990-cin solutions after the expiry date on the product label. Avoid repeated freeze-thaw cycles.
- CL990-cin is sensitive to high concentrations of acids and bases but a short-term exposure to dilute acids can be tolerated.

Quality Control
Each lot is tested to ensure the absence of lot-to-lot variation.
- Endotoxin level: < 1 EU/mg
- Physicochemical characterization (including HPLC, pH, appearance)

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- Cell culture tested: potency validated in CL990-cin -sensitive and CL990-cin -resistant mammalian cell lines
- Non-cytotoxicity of trace contaminants: absence of long-term effects confirmed in CL990-cin -resistant cells

Precautions and Disclaimer

CL990-cin is a harmful compound. Refer to safety data sheet for handling instructions

References

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. 4. 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. 5. Hirose Y. *et al*., 2012. Inhibition of Stabilin-2 elevates circulating hyaluronic acid levels and prevents tumor metastasis. PNAS, 109:
4263 - 4268. 6. 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.
7. 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 .
8. 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. 9. Saxena A. *et al.*, 2002.H2, the minor subunit of the human asialoglycoprotein receptor, trafficks intracellularly and forms homo-oligomers, but does not bind asialoorosomucoid.
J Biol Chem. 277(38):35297-304. 10. 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. 11. Ahmed *et al.*, 2013. TRIFmediated
TLR3 and TLR4 signaling is negatively regulated by ADAM15. J Immunol. 190(5):2217-28. 12. 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. 13. 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. 14. 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. 15. 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. 16. 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. 17. 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. 18. 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. 19. 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. 20. Maue A. *et al.,* 2013. The polysaccharide capsule of Campylobacter jejuni modulates the host immune response. Infect Immun. 81(3):665-72.

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