



Zeocin

Product No.: 900001

Introduction

Zeocin is a selection antibiotic that acts on both eukaryotic and prokaryotic cells. Resistance to Zeocin is conferred by the *Sh ble* gene from *Streptoalloteichus hindustanus*1-3. Zeocin 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.

Product component

10 x 1 ml (1 g). Zeocin is supplied a sterile filtered blue solution at 100 mg/ml in HEPES buffer.

Storage / Stability

- Zeocin is shipped at room temperature. Upon receipt it should be stored at 4°C or at -20 °C. 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.

Note: Zeocin is stable for 1 month at room temperature.

Zeocin is a harmful compound. Refer to safety data sheet for handling instructions.

Quality Control

Each lot is thoroughly tested to ensure the absence of lot-to-lot variation.

- Endotoxin level: < 1 EU/mg
- Physicochemical characterization (including HPLC, pH, appearance)
- Cell culture tested: potency validated in Zeocin-sensitive and Zeocin-resistant mammalian cell lines
- Non-cytotoxicity of trace contaminants: absence of long-term effects confirmed in Zeocin-resistant cells

Instruction

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

Most cells growing aerobically are killed by 0.5 to 1000 µg/ml 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 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 2 to 3 in hypertonic



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media, such as those used for protoplast regeneration. Hence, using low salt medium when possible decreases the amount of Zeocin needed.

■ **Escherichia coli**

The Sh ble gene and the hybrid genes in vectors are driven by synthetic E. coli promoters (i.e. EM7). The cells of the common E. coli recipient strains (i.e. HB101, DH5 α , 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 μ g/ml of Zeocin. Plates containing Zeocin are stable for 1 month when stored at 4°C.

■ **Mammalian cells**

The working concentration of Zeocin for mammalian cell lines varies from 50 to 400 μ g/ml, in a few cases can be as low as 20 μ g/ml or as high as 1000 μ g/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, may 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 on the next page.

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.

Zeocin is normally used at a concentration of 100 μ g/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	Medium	Zeocin conc
B16 (Mouse melanocytes)	RPMI	20-250 μ g/ml
CHO (Chinese hamster ovarian cells)	DMEM	100-500 μ g/ml
COS (Monkey kidney cells)	DMEM	100-400 μ g/ml
HEK293 (Human embryonic kidney cells)	DMEM	100-400 μ g/ml
HeLa (Human uterine cells)	DMEM	50-100 μ g/ml
J558L (Mouse melanocytes)	RPMI	400 μ g/ml
MCF-7 (Human breast adenocarcinoma cells)	DMEM	100-400 μ g/ml
MEFs (Mouse embryonic fibroblasts)	DMEM	200-400 μ g/ml
THP-1 (Human monocytes)	RMPI	200 μ g/ml