



Zeocin

BACKGROUND:

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

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.

CHEMICAL PROPERTIES:

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. Zeocin is freely soluble in water (<500 mg/ml) forming a blue solution.

SAFETY CONSIDERATIONS:

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

METHOD:

Preparation of Zeocin solution

1. Resuspend Zeocin 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 0.22 μ m sterile filter.
3. Store at -20°C for 1 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.

CONDITIONS OF SELECTION:

Most cells growing aerobically are killed by Zeocin in the concentration range of 0.5 to 1000 $\mu\text{g/ml}$. 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 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, 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 25-50 $\mu\text{g/ml}$ 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 $\mu\text{g/ml}$, in a few cases can be as low as 20 $\mu\text{g/ml}$ or as high as 1000 $\mu\text{g/ml}$. In a



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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 on the next page.

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	Medium	Zeocin 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