Zeocin®

Selective antibiotic for the *Sh ble* gene; cell culture tested

Catalog code: ant-zn-05, ant-zn-1, ant-zn-5, ant-zn-5b

http://www.invivogen.com/zeocin

For research use only

Version 22G21-MM

PRODUCT INFORMATION

Contents

Zeocin® is supplied a sterile filtered blue solution at 100 mg/ml in HEPES buffer.

- **ant-zn-05:** 5 x 1 ml (500 mg)
- **ant-zn-1:** 10 x 1 ml (1 g)
- **ant-zn-5:** 50 x 1 ml (5 g)
- **ant-zn-5b:** 1 x 50 ml (5 g)

Storage and 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.
- The expiry date is specified on the product label.
- 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.*

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

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*[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.

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.

SAFETY CONSIDERATIONS

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

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.

CONDITIONS OF SELECTION

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 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 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 25-50 µg/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 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, 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.

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® concentration	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

REFERENCES

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RELATED PRODUCTS

Product	Description	Catalog Code
Other selective antibiotics		
Blasticidin	Selective antibiotic for the *bsr* or BSD genes	ant-bl-05
G418	Selective antibiotic for the *neo* gene	ant-gn-1
Hygromycin B Gold	Selective antibiotic for the *hph* gene	ant-hg-1
Puromycin	Selective antibiotic for the *pac* gene	ant-pr-1
Plasmids encoding the *Sh ble* gene		
pMOD2-Zeo	Plasmid encoding a synthetic *Sh ble* gene	pmod2-zeo
pSELECT-zeo-LacZ	LacZ-expression plasmid selectable with Zeocin®	psetz-lacz
pSELECT-zeo-mcs	Expression plasmid selectable with Zeocin®	psetz-mcs

TECHNICAL SUPPORT
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Asia: +852 3622-3480
E-mail: info@invivogen.com