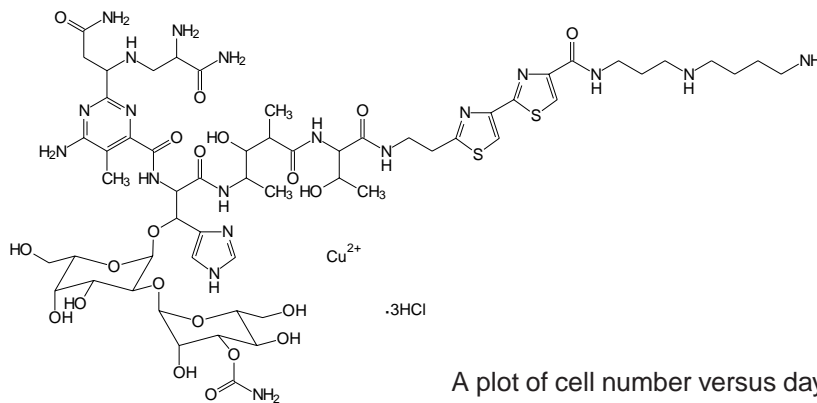


BLEOCIN[™]

A Potent Antibiotic for the Selection of Bleomycin Resistance

The members of the bleomycin family of antibiotics, that includes bleomycin, phleomycin, tallysomyacin, pepleomycin, and Zeocin[™], are DNA-cleaving glycopeptides that exert their toxic effects on prokaryotes and eukaryotes at low concentrations.¹ Bleomycin, complexed with metal ions such as Cu and Fe, intercalates between DNA base pairs and catalyzes the reduction of molecular oxygen to free radicals that can break DNA strands and inhibit further DNA synthesis.² Bleomycin is effective against cycling and non-cycling cells, and is considered to be most toxic in the G2 phase of the cell cycle. These properties make bleomycin a potent therapeutic agent in the treatment of several types of human cancers.

Plasmid-mediated resistance against the bleomycin family of antibiotics is found in some Gram-negative and Gram-positive bacteria. At least three *ble* resistance genes have been characterized. The protein encoded by the *Sh ble* gene from *Streptoalloteichus hindustanus*, reversibly binds to bleomycin in a one-to-one ratio, preventing its intercalation and cleavage of DNA.³ These genes, can be used as dominant markers in mammalian cell lines and bleomycin or Zeocin[™] can be used to select clones from cell lines transfected with the resistance genes. Commercial preparations of bleomycin and Zeocin[™] consist of mixtures of components with differing potencies. BLEOCIN[™] from *Streptomyces verticillus*, is a single component of bleomycin family group A. It consists of a peptide complexed with a disaccharide moiety and copper. We tested the efficacy and potency of BLEOCIN[™] on several wild type and transfected cell lines.



Fresh medium and antibiotics were added every 3 days to quadruplicate wells, and a second replicate set was left undisturbed. On day 5, 10, and 15 the percentage of viable cells was determined by trypan blue exclusion. BLEOCIN[™] killed the Jurkat cells in a concentration-dependent manner. In as little as 32 $\mu\text{g/ml}$ of BLEOCIN[™], complete loss of viability was observed on day 10, and 16 $\mu\text{g/ml}$ BLEOCIN[™] killed all cells by day 15 (Figure 1A). Similar results were obtained regardless whether fresh antibiotic was added or not. No spontaneously resistant colonies appeared at these concentrations after four weeks. At least 128 $\mu\text{g/ml}$ of Zeocin[™] was required for an identical effect (Figure 1B). Live cells were still detected in the wells containing 64 $\mu\text{g/ml}$

Zeocin[™] on day 15. These data show that BLEOCIN[™] is up to 8-fold more potent than Zeocin[™] in killing wild-type Jurkat cells. These same doses of Zeocin[™] and BLEOCIN[™] produced similar effects on cell viability with other cells types (see Table 1).

A plot of cell number versus days of treatment shows that at doses lower than 512 $\mu\text{g/ml}$, Zeocin[™] allowed for early cell growth. In contrast, BLEOCIN[™] killed cells more quickly and no cell growth was observed at the effective doses (Figures 2A & 2B).

Transfection of cells with *Sh ble* gene confers a 15-fold increase in resistance to BLEOCIN[™].

In another experiment, Jurkat Human T Leukemia cells (5×10^6) were transfected with 15 μg of pcDNA3.ZEO plasmid vector (Invitrogen) by electroporation. After 36 hours, cells were plated at 10,000 cells/well in flat bottom 96-well plates. The cells were selected in 250 $\mu\text{g/ml}$ Zeocin[™] and two individual clones, denoted F9 and B4, were chosen for further studies. The two clones were plated at 10,000 cells/well in various concentrations of Zeocin[™] or BLEOCIN[™]. Fresh medium and antibiotic were added on day 5. The growth and viability was assessed on days 7 and 14. Both clones grew well in 125 $\mu\text{g/ml}$ BLEOCIN[™] with only a slight decrease in viability. The F9 were completely killed at 250 $\mu\text{g/ml}$ BLEOCIN[™], however, some B4 cells remained viable at this

Mammalian cell lines are sensitive to low concentrations of BLEOCIN[™].

Jurkat Human T Leukemia cells were plated in a 96-well plate at 1×10^4 cells per well in RPMI 1640 medium with 10% FBS. Zeocin[™] or BLEOCIN[™] were added at concentrations ranging from 4 to 1024 $\mu\text{g/ml}$ for up to 15 days.

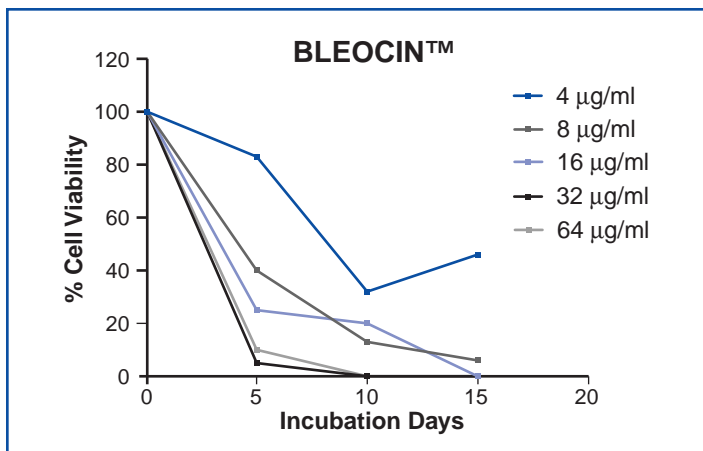


Figure 1A.

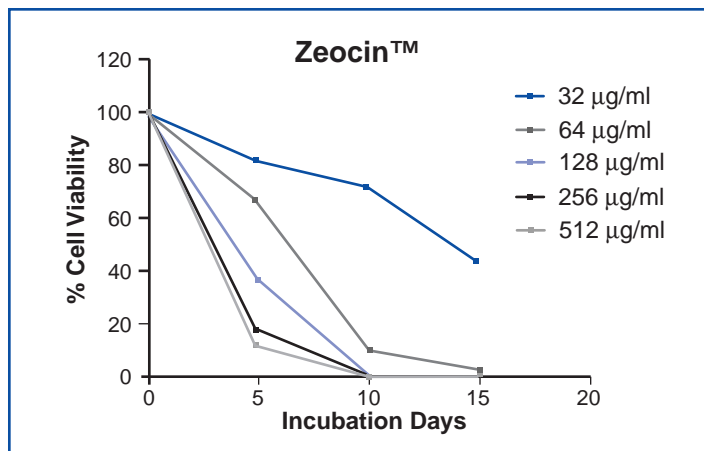


Figure 1B.

dose (Figure 3A). Since wild-type cells grow in 4 to 8 µg/ml of BLEOCIN™ but are killed at 16 µg/ml, transfection conferred an approximate 15-fold increase in resistance to this drug. Both clones grew well in 1000 µg/ml of Zeocin™, however, F9 cells were killed at 2000 µg/ml (Figure 3B). The wild-type cells grew in 64 µg/ml Zeocin™ but were killed in 128 µg/ml; once again transfection conferred an approximate 15-fold increase in resistance. These data clearly demonstrate that BLEOCIN™ is much more potent than Zeocin™, yet it confers the same therapeutic window in terms of resistance.

Low doses of BLEOCIN™ can be used to select and maintain transfectant clones.

To further demonstrate the suitability of BLEOCIN™ as a selection agent, 5×10^6 Jurkat cells were transfected with 15 µg of the pcDNA3.1ZEO vector. Clones were selected in the presence of 125 and 250 µg/ml Zeocin™ or 16 and 32 µg/ml BLEOCIN™. The number of positive clones emerging with each antibiotic was assessed over a 4 week period. Fresh medium was added to each well on days 6, 14, 18, 22, and 26, and fresh antibiotics were added on days 6, 18, and 26. Clones began to emerge on day 17. Aliquots of the positive clones were transferred into wells containing fresh medium and antibiotics to confirm drug resistance. Fewer clones emerged at the higher concentrations of both antibiotics and the growth was slower; however, similar numbers of colonies were obtained when BLEOCIN™ was used at one eighth the concentration of Zeocin™ (Figure 4).

Table 1.

Cell Line	Species	Medium	BLEOCIN™
Jurkat	Human	RPMI 1640, 10% FBS	16 - 32 µg/ml
3T3	Mouse	RPMI 1640, 10% FBS	< 50 µg/ml
BHK	Hamster	DMEM, 10% FBS	< 30 µg/ml
MCF-7	Human	DMEM, 10% FBS	15 - 20 µg/ml
Sf9	<i>Spodoptera frugiperda</i>	Sf900 II, Serum free	16 - 32 µg/ml
HEPG2	Human	MEM, 10% FBS	16 - 32 µg/ml

These data demonstrate that:

- BLEOCIN™ is an extremely effective antibiotic that can be used to select clones carrying a bleomycin family resistance gene.
- Like Zeocin™ and phleomycin, BLEOCIN™ is toxic to prokaryotic and eukaryotic cells including mammalian, yeast, and insect cells.
- BLEOCIN™ is 8-fold more potent than Zeocin™ and works at concentrations similar to phleomycin.¹
- Additional antibiotic is not required during the selection process.
- The low concentrations of BLEOCIN™ required for selection make it less expensive to use.
- The fact that BLEOCIN™ is provided as a single component rather than a mixture, has the additional advantage of a consistent potency that leads to more reliable and reproducible results.

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References:

1. Mulsant, P., et al. 1988. *Somatic Cell and Molecular Genetics* **14**, 243.
2. Ehrenfeld, G.M., et al. 1987. *Biochemistry* **26**, 931.
3. Gatignol, A., et al. 1988. *FEBS Letters* **230**, 171.

BLEOCIN™ is a trademark of CALBIOCHEM®
Zeocin™ is a trademark of Invitrogen Corp.

BLEOCIN™

Cat. No. 203408

100 mg

250 mg

Ask us about sample sizes of BLEOCIN™

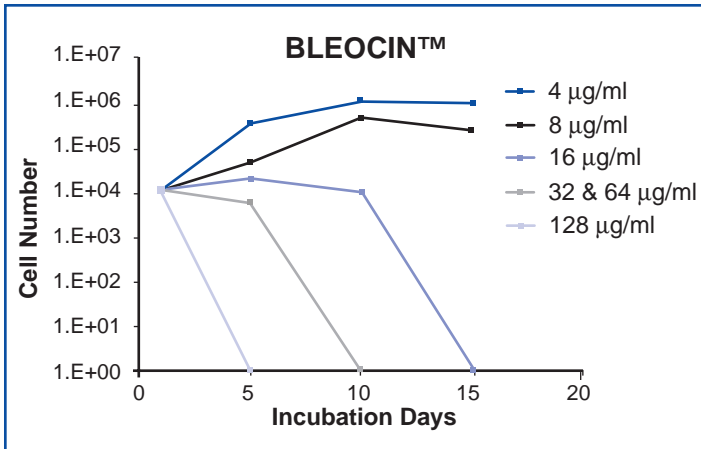


Figure 2A.

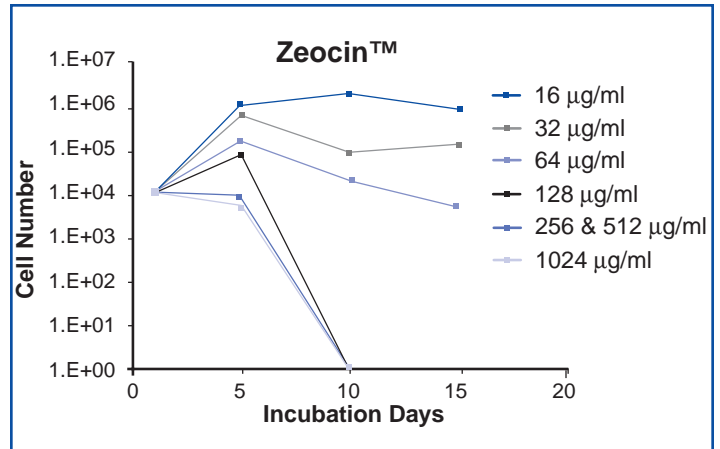


Figure 2B.

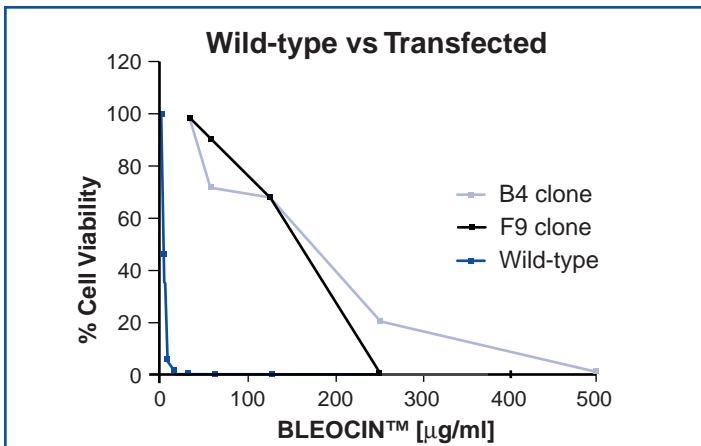


Figure 3A.

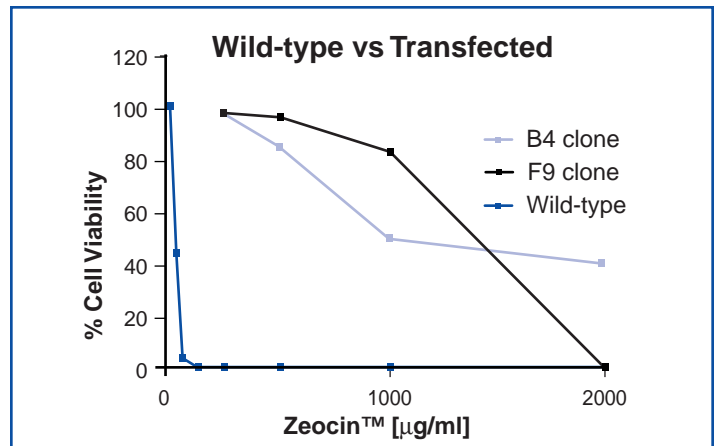


Figure 3B.

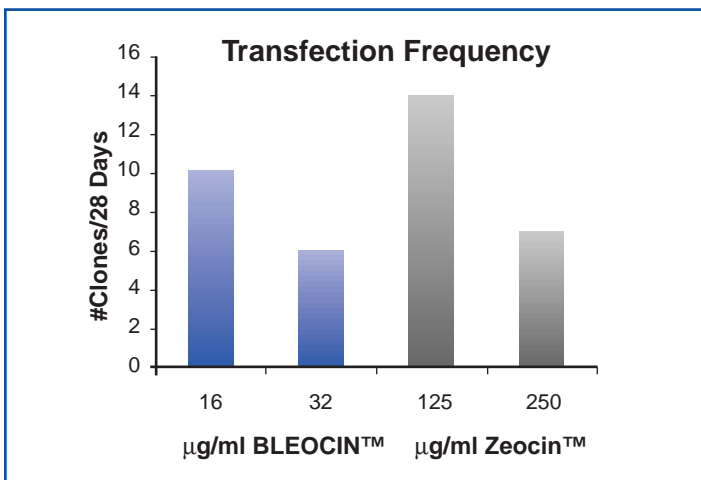


Figure 4.

We have a complete line of antibiotics for selection of genetic markers.



