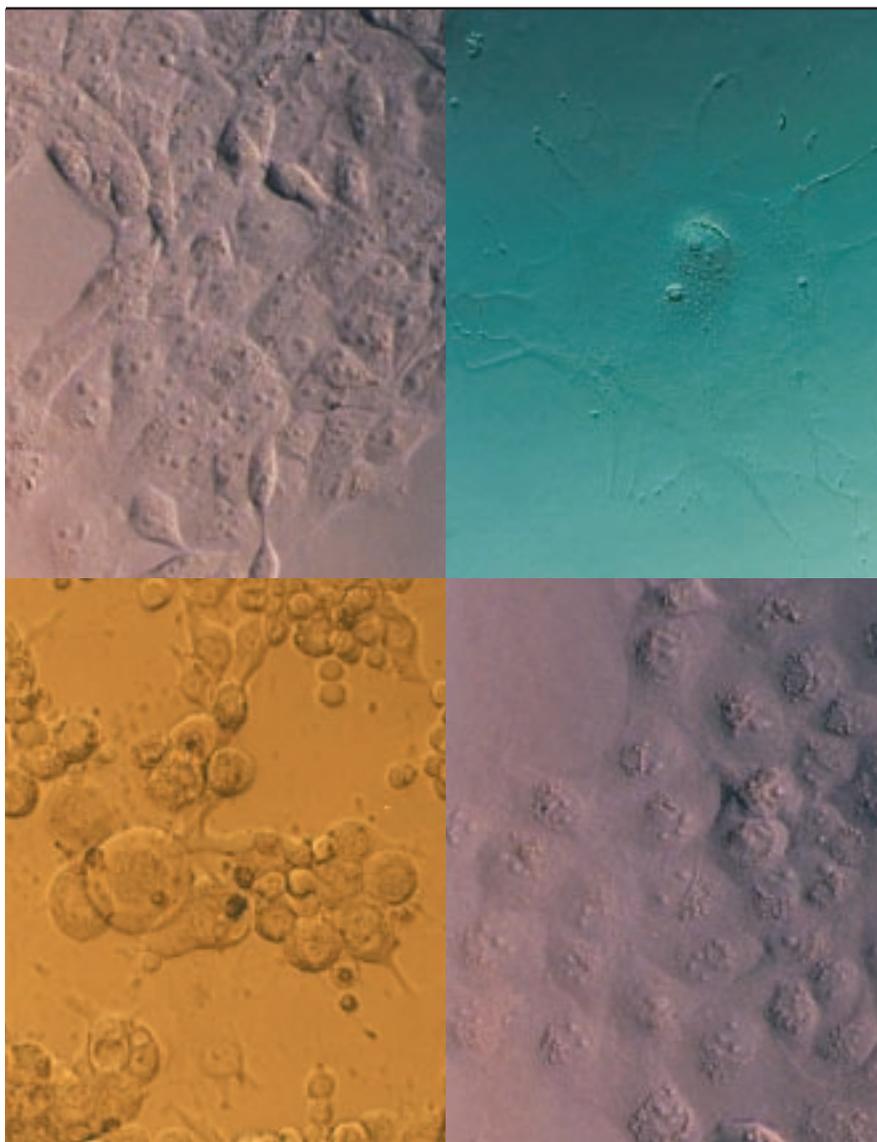




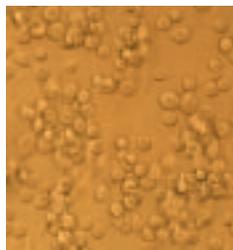
Potent, reliable selection reagents for gene expression



Selection reagents for prokaryotic and eukaryotic expression hosts provide:

- Fast, potent selection of stable cell lines
- Cost-effective selection
- Effective selection in multiple organisms

Selective antibiotics for all your needs



Invitrogen offers a wide variety of top-quality selection reagents to complement its eukaryotic and prokaryotic expression vectors. To ensure effective selection in different cell lines, these selection reagents can be used to address the following needs:

- Fast selection of stable eukaryotic cell lines
- Cost-effective selection
- Selection in multiple organisms
- Dual selection

Geneticin®: The industry standard for eukaryotic cell selection

Geneticin® is the most cited antibiotic in history, and the most trusted and reliable antibiotic used by scientists worldwide (Figure 1). Geneticin® is powerful, reliable, efficient, and cost effective. Accept no substitutes! Toxic to bacteria, yeast, protozoans, helminths and higher plant and mammalian cells, Geneticin® is well-suited for selection of eukaryotic cell lines. Resistance to Geneticin® is conferred by the neomycin resistance genes which are dominant and are located on both transposons Tn601 (903) (aminoglycoside phosphotransferase 3'(I) or APH (3')I) and Tn5

(aminoglycoside phosphotransferase 3'(II) or APH (3')II). While the genes are bacterial in derivation, they can be expressed in eukaryotic cells for selection purposes. Introduction of either of these genes confers resistance to Geneticin® Selection Reagent. Geneticin® interferes with the function of 80S ribosomes and blocks protein synthesis in eukaryotic cells. Resistant cell lines can be generated in 10 to 14 days.

Formula: $C_{20}H_{40}N_4O_{10} \bullet 2H_2SO_4$
 FW: 496.6•196.1 g/mole

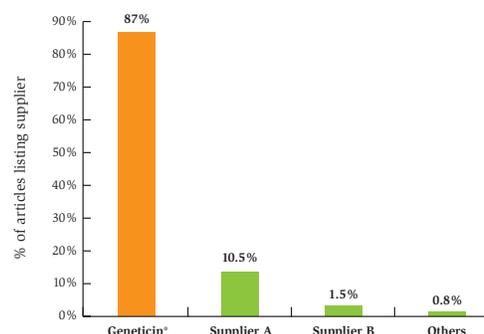
Enjoy the convenience of Liquid Geneticin®

For the ultimate in convenience, Geneticin® is available in a calibrated, higher potency, liquid format. Eliminate the time, effort, and potential errors of producing sterile working stock from powder. Each lot is supplied at 50 mg/ml active concentration and is tested in an *in vitro* cell culture assay to ensure performance and validate potency. Liquid Geneticin® provides 100% activity vs. 75% with the powder format.

Liquid Geneticin® provides:

- Higher potency than powder (100% vs. 75%)
- Ease-of use: no calculating, weighing, filtering or aliquoting
- Minimal user effort: no sterilizing filters and receivers necessary
- Consistent potency: each lot is supplied at 50 mg/ml active concentration

Figure 1 - Geneticin® versus G418 antibiotic citations in scientific journals worldwide



Blasticidin S: Only six days to stable mammalian cell lines

Blasticidin S is such a potent antibiotic that concentrations of 10 µg/ml or less will cause rapid cell death of non-resistant cells (Figure 2). You can establish stable mammalian cell lines in less than one week. In addition, the extremely low effective concentrations of Blasticidin S make it a very cost-effective selection reagent. A nucleoside antibiotic isolated from *Streptomyces griseochromogenes*, Blasticidin S is a potent translational inhibitor in both prokaryotic and eukaryotic cells. Resistance is conferred by the *bsd* gene product from *Aspergillus terreus*. Cells sensitive to Blasticidin clump together, round up, and detach from the plate.

Formula: $C_{17}H_{26}N_8O_5$ HCl

FW: 458.9 g/mole

Zeocin™: A novel mode of action

Zeocin™ is highly effective in a variety of organisms, including mammalian and insect cell lines, as well as in yeast, bacteria, and plants. As a member of the bleomycin family, Zeocin™ causes cell death by intercalating into and cleaving DNA. Resistance to Zeocin™ is conferred by the *Sh ble* gene product, which binds the antibiotic and prevents it from binding DNA. This selection agent is effective in multiple cell types, so eukaryotic expression vectors only need to carry one drug selection marker. This reduces the overall size of the vector and makes subcloning and transfection easier and more efficient.

Figure 2 - Blasticidin S sensitivity curves for HeLa cells

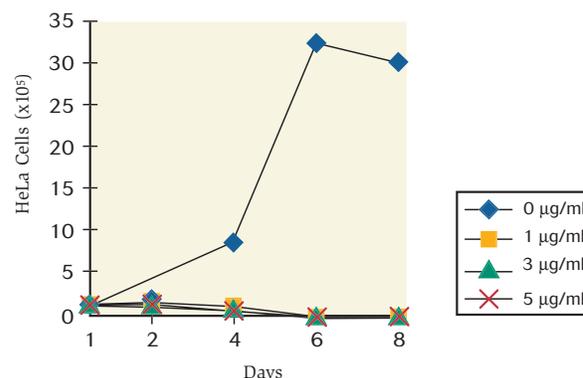
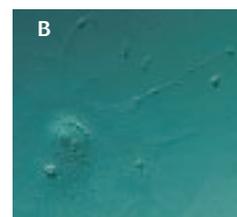


Figure 3 - COS-1 Cells under Zeocin™ selection



COS-1 cells without selection



COS-1 cells selected with 400 µg/ml Zeocin™ for four days

Formula: $C_{55}H_{83}N_{19}O_{21}S_2Cu$

FW: 1137.41 g/mole

Hygromycin B: Double up perfectly

Hygromycin B is an aminoglycosidic antibiotic that inhibits protein synthesis by disrupting translocation and promoting mistranslation at the 80S ribosome. Because it uses a different mode of action than Geneticin®, Blasticidin S, or Zeocin™, it's perfect for

dual-selection experiments when used in conjunction with another selection agent.

Formula: $C_{20}H_{37}N_3O_{13}$

FW: 527.5 g/mole

Prokaryotic selection reagents

The following antibiotics are ideal for prokaryotic selection. The corresponding markers are present in a wide variety of Invitrogen vectors.

Antibiotic	Formula	FW
<p>Ampicillin</p> <p>Ampicillin is a semi-synthetic penicillin derived from the penicillin nucleus, 6-amino-penicillanic acid. It causes cell death by inhibiting cell wall biosynthesis. Resistance to ampicillin is mediated by β-lactamase cleavage of the β-lactam ring (<i>bla</i> gene).</p>	$C_{16}H_{18}N_3O_4 \text{ SNa}$	371.4 g/mole
<p>Kanamycin Sulfate</p> <p>Kanamycin is effective as a bacteriocidal agent by inhibiting ribosomal translocation and eliciting miscoding. Resistance is conferred by the Kan^R-Tn5 gene product (aminoglycoside phosphotransferase), which modifies the antibiotic and prevents interaction with ribosomes. Liquid kanamycin (100X) contains 10 mg/ml kanamycin (base) utilizing kanamycin sulfate in 0.85% saline.</p>	$C_{18}H_{36}N_4O_{11} \bullet H_2SO_4$	583 g/mole
<p>Tetracycline</p> <p>Tetracycline is a bacteriocidal agent that inhibits protein synthesis by preventing binding of aminoacyl-tRNA to ribosomes. Resistance is conferred by the Tet^R-Tn10 gene product (an inner membrane protein that effluxes the antibiotic), which blocks cell wall permeability.</p>	$C_{22}H_{24}N_2O_8 \bullet HCl$	480.9 g/mole
<p>Cefotaxime</p> <p>Cefotaxime is a member of the cephalosporin antibiotic class of drugs, and has a wide spectrum of activity. Cefotaxime works by inhibiting bacterial cell wall biosynthesis.</p>	$C_{16}H_{16}N_5O_7S_2Na$	477.4 g/mole
<p>Carbenicillin</p> <p>Carbenicillin sodium is a semi-synthetic penicillin antibiotic which interferes with final cell wall synthesis of susceptible bacteria. It is commonly used in place of ampicillin, reducing the occurrence of satellites.</p>	$C_{17}H_{16}N_2O_6S_2Na$	422.4 g/mole
<p>Actinomycin D</p> <p>Actinomycin D is an antineoplastic antibiotic which inhibits DNA-primed RNA polymerase by complexing with DNA via deoxyguanosine residues.</p>	$C_{62}H_{86}N_{12}O_{16}$	1,255 (anhydrous) g/mole
<p>Streptomycin Sulfate</p> <p>Streptomycin sulfate, an aminoglycoside used for bacterial selection, binds to the small ribosomal subunit and interferes with protein biosynthesis.</p>	$(C_{21}H_{39}N_7O_{12})_2 \bullet 3H_2SO_4$	1,457 g/mole

Selection Reagents to fit your needs

Eukaryotic selection agents and resistant vectors

With four different selection agents available for eukaryotic cells, there's sure to be one to meet your research needs. Table 1 describes the selection conditions for each agent in several different cell types, and also the applications supported by Invitrogen vectors carrying the appropriate resistance gene.

Table 1 - Selection agents and resistant vectors

Selection agents	Cell types	Selection conc. (µg/ml)*	Invitrogen Vectors carrying resistance marker
Blasticidin S	HeLa	1-3	• pcDNA6 vectors (Constitutive mammalian expression)
	NIH3T3	5-10	• BsdCassette™ vectors (Constructing customized Blasticidin-resistant vectors)
	CHO	5-10	
	COS-1	3-10	• pIB/V5-His-TOPO® vectors (Stable insect expression)
	293 HEK	5-10	• pMIB/V5-His vectors (Secreted insect expression)
	S2 <i>Drosophila</i>	~ 5	• pCoBlast (Selection vector for DES®)
	<i>S. cerevisiae</i>	~ 25	
Zeocin™	HeLa	~ 150	• pcDNA4 vectors (Constitutive mammalian expression)
	NIH3T3	~ 400	• T-REx™ System, pcDNA4/TO vectors (Inducible mammalian expression)
	CHO	~ 250	• pSecTag2 vector (Secreted mammalian expression)
	COS-1	~ 400	• ZeoCassette™ vectors (Constructing Zeocin™-resistant vectors)
	293 HEK	200-400	• InsectSelect™ System, pIZ/V5-His vectors (Stable expression in insect cells)
	Jurkat T cell	~ 200	
	Sf9 insect	~ 250	• pPICZ and pICZα vectors (Inducible expression in <i>Pichia pastoris</i>)
	S2 <i>Drosophila</i>	~ 75	• pGAPZ and pGAPZα vectors (Constitutive expression in <i>Pichia pastoris</i>)
	<i>Pichia pastoris</i>	~ 100	
<i>S. cerevisiae</i>	200-300		
Geneticin®	HeLa	200-400	• pcDNA3.1™ vectors (Constitutive mammalian expression)
	NIH3T3	600-1,000	• pIND vectors (Ecdysone-Inducible mammalian expression)
	CHO	~ 400	• pShooter™ vectors (Intracellular protein targeting)
	293 HEK	600-800	• pDisplay™ vectors (Protein display)
	Jurkat T cell	600-700	• pVP22 vectors (Protein translocation) • pBlue-TOPO®, pGlow-TOPO® vectors (Assessing promoter activity)
Hygromycin B	HeLa	~ 550	• pcDNA5 vectors (Constitutive mammalian expression)
	CHO	~ 250	• pIND/Hygro vector (Ecdysone-Inducible mammalian expression)
	Jurkat T cell	~ 1,000	• pSecTag2/Hygro vector (Secreted mammalian expression)
	S2 <i>Drosophila</i>	200-300	• pREP vectors (Episomal mammalian expression) • pCoHygro (Selection vector for DES®)

* The optimal concentration for selection of your cell line should be determined using kill curves.

Ordering information

Product	Quantity	Cat. no.
Eukaryotic Selection Reagents		
Geneticin® (powder)	1 g	11811-023
	5 g	11811-031
	25 g	11811-098
Liquid Geneticin® (50 mg/ml solution)	20 ml	10131-035
	100 ml	10131-027
Blasticidin S HCl	50 mg	R210-01
Zeocin™ (100 mg/ml solution)	1 g	R250-01
	5 g	R250-05
Hygromycin B (50 mg/ml solution)	20 ml	10687-010
Mycophenolic Acid	500 mg	11814-019
Prokaryotic Selection Reagents		
Ampicillin (sodium salt, lyophilized)	20 mg	11593-019
Actinomycin D	5 mg	11805-017
Carbenicillin (disodium salt)	5 g	10177-012
Cefotaxime (sodium salt)	2 g	10213-015
Kanamycin Sulfate (powder)	5 g	11815-024
	25 g	11815-032
Kanamycin Sulfate (100X), liquid	100 ml	15160-054
Streptomycin Sulfate	100 g	11860-038
Tetracycline	5 g	Q100-19

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