CALBIOCHEM®

10394 Pacific Center Court
San Diego, CA 92121
(858) 450-9600

Customer Service: (800) 854-3417
Technical Service: (800) 628-8470
Fax: (800) 776-0999

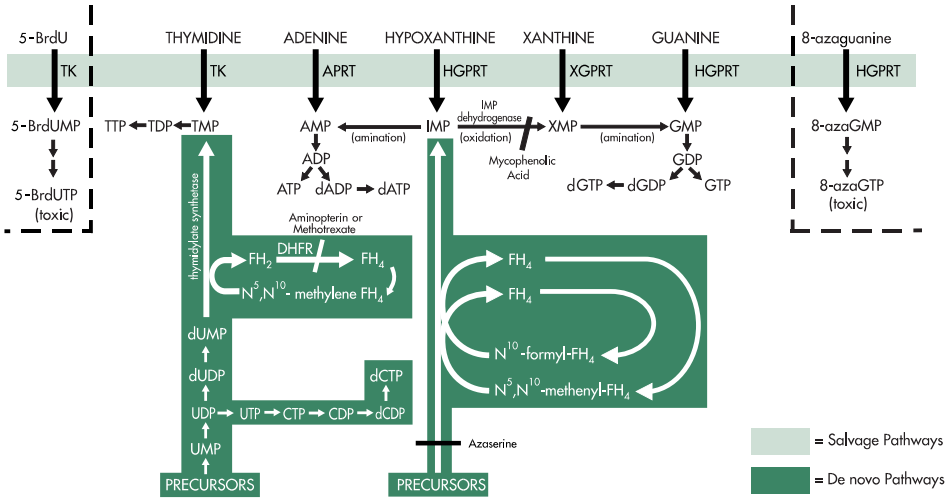
Drugs for Selection of Genetic Markers

REAGENTS FOR POSITIVE AND NEGATIVE SELECTION OF GENES INVOLVED IN NUCLEOTIDE METABOLISM

Techniques for generating somatic cell hybrids, including hybridoma cell lines for monoclonal antibody production, as well as those for generating cell lines containing stably-integrated DNA, frequently employ drugs that inhibit nucleotide biosynthesis. Cells produce ribonucleotides and deoxyribonucleotides via two pathways–*de novo* synthesis or salvage synthesis (see figure below). *De novo* synthesis is the assembly of nucleotides from simple compounds such as amino acids, sugars, CO_2 and NH_3. The first nucleotides produced by this pathway, and the precursors for all other pyrimidine and purine nucleotides, respectively, are UMP and IMP. *De novo* synthesis of IMP and TMP requires tetrahydrofolate (FH4) derivatives as cofactors. The antifolates, aminopterin and methotrexate, block *de novo* synthesis of these nucleotides by inhibiting dihydrofolate reductase (DHFR), the enzyme that regenerates tetrahydrofolate depleted during the conversion of dUMP to TMP. Azaserine blocks only *de novo* purine synthesis by inhibiting glutamine-dependent amination reactions. Salvage synthesis refers to enzymatic reactions that convert free, preformed purine bases or thymidine to their corresponding NMPs. When *de novo* synthesis is blocked, salvage enzymes enable the cell to survive as long as the medium contains appropriate ready-formed purines and/or thymidine. Mammalian cells normally express several salvage enzymes: hypoxanthine-guanine phosphoribosyltransferase (HGPRT), which converts hypoxanthine to IMP and guanine to GMP; adenine phosphoribosyltransferase (APRT), which converts adenine to AMP; and thymidine kinase (TK), which converts thymidine to TMP. The drugs 5-BrdU and 8-azaguanine select for loss of TK and HGPRT, respectively, since these enzymes convert the analogs to their corresponding 5´-monophosphates, enabling their subsequent, and ultimately lethal, incorporation into DNA.

Typically, in the production of murine hybridomas, B lymphocytes from an immunized mouse are fused to non-antibody-producing myeloma cells that are HGPRT-deficient due to preselection to 8-azaguanine- or 6-thioguanine-resistance. Hybridoma cells are then selected in medium containing an inhibitor of *de novo* synthesis (aminopterin in HAT medium; methotrexate in HMT medium; azaserine in AzaH medium) plus the necessary purine and/or pyrimidine salvage precursors (i.e. hypoxanthine and thymidine in HAT or HMT media; hypoxanthine in AzaH medium). After a short time in culture, primary lymphocytes die naturally, while myeloma cells are killed due to blockage of both the *de novo* and salvage pathways. However, hybridoma cells continue to grow because they have the intact salvage pathways of primary lymphocytes plus the immortality of the myeloma cells.

Genes encoding two salvage enzymes, xanthine-guanine phosphoribosyltransferase (XGPRT) and TK, are also used as markers for integration of exogenous DNA into cellular and viral genomes. XGPRT, the product of the *E. coli gpt* gene, is the bacterial counterpart of the mammalian salvage enzyme HGPRT. Unlike HGPRT, however, XGPRT also efficiently converts xanthine to XMP, the immediate precursor to GMP. Thus, if xanthine is provided as a precursor, the XGPRT gene can be used as a dominant selectable marker for resistance to mycophenolic acid, which inhibits conversion of IMP to XMP. For XGPRT selection, adenine or hypoxanthine are added as AMP precursors; usually, aminopterin and thymidine are also included for optimal selection. TK selection is quite versatile because cells can be selected for either loss or acquisition of this gene. HAT medium is used for positive selection of TK (in TK^- parental cells), whereas 5-BrdU, which is toxic in the presence of TK, is used to select for loss of TK. TK^- selection has proven useful for generation of cellular and viral gene knockouts.



Drugs for the Selection of Genetic Markers
Reagents for Positive Selection of Plasmid-Encoded Drug-Resistance

Product	Cat. No.	M.W.	Description	Stock Solution[a]	Suggested Conc. For Selection[b]	Refs
Ampicillin, Sodium Salt	171254	371.4	Penicillin derivative that inhibits bacterial cell wall synthesis. Used for the selection of the bacterial *amp*^{r} gene, whose product, β-lactamase, cleaves the drug's β-lactam ring.	H_2O (25-50 mg/ml)	30-50 µg/ml (*E. coli*)	1,2
Blasticidin S, Hydrochloride	203350	458.9	Nucleoside antibiotic that inhibits eukaryotic and prokaryotic translational elongation. Used for selection of the *bsr* gene (*B. cereus*), whose product, blasticidin S deaminase, inactivates blasticidin S by converting it to a deaminohydroxy derivative.	H_2O (5-10 mg/ml) Avoid Freeze/Thaw	2-10 µg/ml (mammalian)	3-5
BLEOCIN™	203408	1613.5	A high purity, potent glycoprotein antibiotic of the bleomycin family. Can be used at low concentrations for selection of cells containing the *ble* gene, whose product inactivates antibiotics of the bleomycin family.	H_2O (16-32 mg/ml)	16-32 µg/ml (mammalian)	6-8
Carbenicillin, Disodium Salt	205805	422.4	Penicillin derivative that can also be used to select for *amp*^{r} plasmids in bacteria.	H_2O (25-50 mg/ml)	30-50 µg/ml (*E. coli*)	1
Chloramphenicol	220551	323.1	Inhibits bacterial protein synthesis by binding to the 50S ribosomal subunit, blocking the peptidyl transferase reaction. Used for selection of the bacterial *cat* gene, whose product, chloramphenicol acetyltransferase, inactivates the drug via acetylation.	H_2O (2.5 mg/ml) or EtOH (35 mg/ml)	10-30 µg/ml (*E. coli*)	1, 2
G 418 Sulfate	345812	692.7	Aminoglycoside that inhibits prokaryotic and eukaryotic protein synthesis. Used for the selection of the bacterial *neo*^{r}/*kan*^{r} gene, whose product, aminoglycoside 3´-phosphotransferase, inactivates G 418, neomycin, and kanamycin by phosphorylation.	H_2O or culture medium (50 mg/ml, active drug)	0.1- 2.0 mg/ml active drug (mammalian)	1, 9-11
G 418, Sterile-Filtered Solution	345812	692.7	See above.	H_2O as supplied (50 mg/ml active)	0.1- 2.0 mg/ml active drug (mammalian)	-
L-Homoserine	38586	119.1	Common free amino acid in plants that are toxic to mammalian cells at concentrations greater than 1 mM. Used in conjunction with threonine-depleted media for the selection of mammalian cells transfected with pSVthrBC which codes for homoserine kinase and threonine synthase. Thus allowing the mammalian cells to produce the essential threonine.	H_2O (1.19 mg/ml)	5-25 mM (mammalian)	12
Hygromycin B	400051	527.5	Aminoglycoside that inhibits prokaryotic and eukaryotic translation by disrupting ribosome translocation and inducing mRNA misreading. Used to select for the *E.coli hph* gene, whose product, hygromycin B phosphotransferase, inactivates the drug by phosphorylation.	H_2O as supplied (~ 400 mg/ml) or ≥ 50 mg/ml in H_2O or culture medium.	0.05-1.0 mg/ml (mammalian)	1, 9 11,13
Kanamycin Sulfate	420311	582.6	Aminoglycoside that inhibits bacterial translation by binding to the 30S ribosomal subunit, causing mistranslocation. Used for selection of the bacterial *neo*^{r}/*kan*^{r} gene.	H_2O (10-25 mg/ml)	30-50 µg/ml (*E. coli*)	1, 2
Puromycin, Dihydrochloride	540222	544.4	Nucleoside that resembles the aminoacyl-adenylyl terminus of aminoacyl-tRNA and competes for binding to the large ribosomal subunit. Its incorporation into a growing polypeptide causes termination. Used for selection of the *pac* gene (*S. alboniger*), whose product, puromycin acetyltransferase, inactivates the drug via acetylation.	H_2O (5-50 mg/ml)	1.0-30 µg/ml (mammalian)	14,15
Streptomycin Sulfate	5711	1457.7	Aminoglycoside that inhibits protein synthesis by binding to the 30S ribosomal subunit. Used for selection of the *stm* gene, whose product, an aminoglycoside phosphotransferase, inactivates the drug via phosphorylation.	H_2O (10-50 mg/ml)	30-50 µg/ml (bacteria)	1, 2
Tetracycline, Hydrochloride	58346	480.9	Inhibits bacterial translation by binding to the 30S ribosomal subunit, preventing binding of aminoacyl tRNA to the "A site" and blocking translation. Used for selection of the bacterial *tet*^{r} gene, whose product functions in active export of the drug from the cell. Can also be used in mammalian cells in conjunction with tetracycline inducible promoters.	H_2O or 1:1 H_2O:EtOH (12-20mg/ml) Protect from light.	12.5-15 µg/ml	1, 2
Thiostrepton	598226	1664.8	Thiazole-containing peptide that inhibits translational elongation by binding to the GTPase center of the large subunit ribosomal RNA. Used primarily for selection, in bacteria of *Streptomyces* sp., of the *tsr* gene (*S. azureus*), which encodes a 23S rRNA methylase whose action prevents thiostrepton from binding to ribosomes.	DMSO (10 mg/ml) Unstable. Prepare fresh and protect from light.	10-50 µg/ml (*Streptomyces*)	16,17

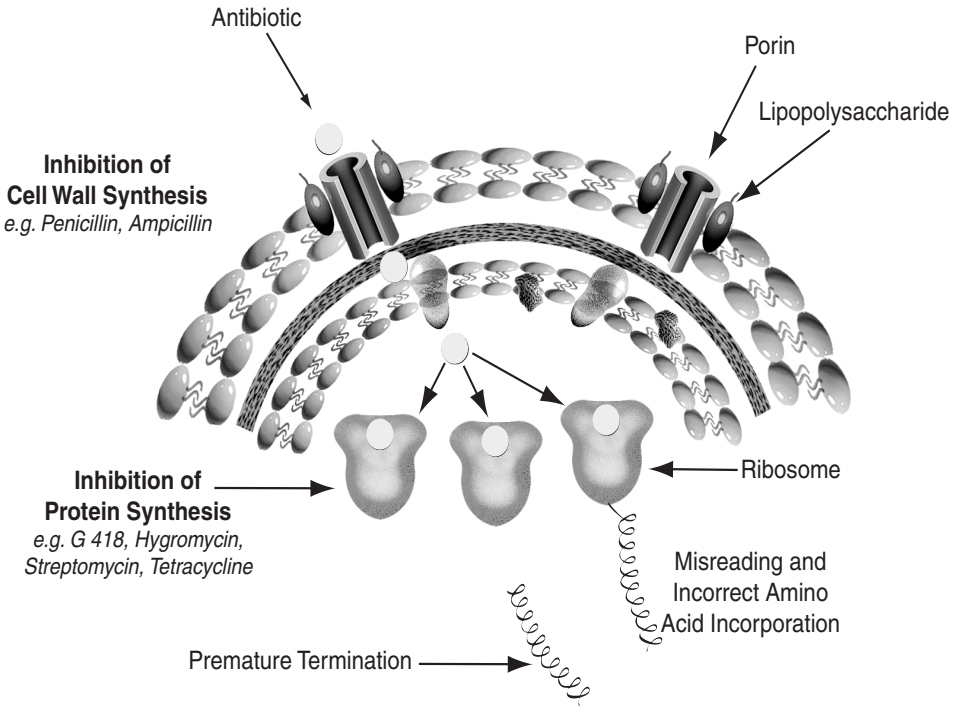
Drugs for Genetic Selection

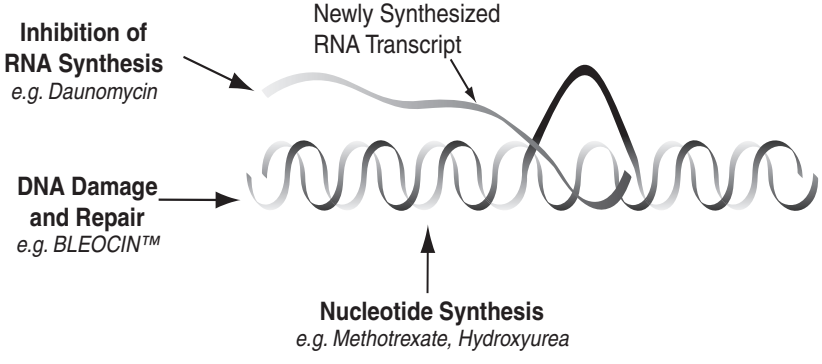
Antibiotics can be classified into several groups by their mechanism of action. These include inhibition of bacterial wall, protein, RNA, and DNA synthesis. They can also interfere with essential cell processes such as microtubule function. Antibiotics are used to give the desired organism a selective advantage over the undesired organism. For example, antibiotics such as ampicillicin which inhibits cell wall synthesis, selectively destroy bacterial cells over mammalian cells since mammalian cells lack a cell wall. Other antibiotics such as bleomycin, which damages DNA and prevents repair, act on all cells that possess DNA. These types of antibiotics are more toxic to rapidly dividing cells, such as neoplasms. However, they also exert their toxic effects on other mammalian cells.

Mechanisms by which cells become antibiotic-resistant include inactivation of the antibiotic, mutation of the drug target, removal by efflux, and over expression of the drug target. Examples of these mechanisms include the *amp*^{r} gene that codes for β-lactamase and the *ble* gene that codes for bleomycin resistance. These genes encode proteins that inactivate the drug. Alternatively, in the case of paclitaxel resistances the multi-drug-resistance (*mdr*) gene is over-expressed; the expressed *mdr* protein pumps out the toxic drug. Although resistance is a growing problem in medical treatment, molecular biologists routinely exploit these resistance genes to select out populations of cells which express a gene of interest. In general, a plasmid is created which contains both the gene of interest, and the resistance gene. Cells are transfected with the plasmid, allowed to recover, and treated with the antibiotic corresponding to the expressed resistance gene. The sensitivity of the cell population to antibiotics depends on several factors. These include the rate of division of the cell, the baseline level of toxicity and whether or not the parent (untransfected) cell line has become resistant. Many commonly-used cell lines have become resistant to multiple antibiotics usually by acquiring a resistance plasmid. In addition, they are very susceptible to mutations that may lead to resistance.

The length of time required to select cells is an important factor. The longer cells are exposed to antibiotics, the more likely that resistant populations will emerge that, may not contain the gene of interest. For example selection with BLEOCIN™, a highly toxic compound, will be faster and more specific than selection with other types of drugs. Due to the number of variables, it is best to perform a kill-curve on your cell line of interest with the antibiotic that is to be used. A rough kill-curve can be generated by plating identical numbers of cells in dishes, or wells, of a cell culture plate and incubating with increasing concentrations of antibiotic for approximately two weeks.

CALBIOCHEM® offers a wide variety of antibiotics for cell selection as well as for other purposes. We strive to provide the highest quality antibiotics for the best value. This figure classifies antibiotics by their mechanism of action.





Other Antibiotics Available from CALBIOCHEM®

Antibiotic	Description	Cat. No.
Aclacinomycin A (Aclarubicin)	Anthrocyclin anti-tumor agent. Inhibits topoisomerase I/II and the degradation of ubiquinated proteins.	112270
Actinomycin D (Dactinomycin)	Anti-neoplastic which inhibits DNA-primed RNA polymerase by complexing with DNA.	114666
Amikacin	Aminoglycoside; inhibits protein synthesis by binding to the bacterial 30S ribosomal subunit.	129880
Antimycin A	Inhibits mitochondrial ATP synthase.	1782
Bacitracin (Altracin)	Antibiotic and peptidase inhibitor. Inhibits bacterial cell wall synthesis.	1951
Cefotaxime	Inhibits bacterial cell wall synthesis. β-lactamase resistant.	219380
Chlortetracycline (Aureomycin)	Anti-bacterial. Inhibits protein synthesis through binding to the 30S ribosomal subunit. Selective for bacteria due to a prokaryotic transport system that imports the drug.	220725
Chromomycin A_3 (Toyomycin)	Antibiotic that inhibits RNA synthesis through inhibition of DNA-dependent RNA polymerase. Flourescent.	230752
Coformycin	Inhibitor of adenosine deaminase.	234108
Cycloheximide	Anti-fungal. Inhibits protein synthesis in eukaryotes but not prokaryotes through interaction with translocase.	239764
Daunorubicin (Daunomycin)	Inhibits RNA and DNA synthesis. Also inhibits topoisomerase I and II.	251800
Doxorubicin (Adriamycin)	Anti-neoplastic. Inhibits topoisomerase II. Intercalates with DNA. Also interacts with cytochrome p450 to generate free radicals.	324380
Epirubicin	Anti-neoplastic. Inhibits topoisomerase II. Intercalates with DNA. Also interacts with cytochrome p450 to generate free radicals.	324905
Gentamycin Sulfate	Aminoglycoside; inhibits bacterial protein synthesis.	345814
Gramicidin A	Pentadecapeptide antibiotic. Renders membranes permeable to protons and alkali metal ions.	368020
Minocycline	Anti-bacterial. Inhibits bacterial protein synthesis by binding to the 30S bacterial ribosome. Also inhibits MMP-3.	475843
Mitomycin C	Anti-neoplastic which inhibits DNA synthesis and cross-links DNA at guanine and adenosine residues.	475820
Neomycin Sulfate	Aminoglycoside; inhibits bacterial protein synthesis. Also blocks voltage-sensitive Ca^{2+} channels.	480100
Nocodazole	Has anti-microtubular activity.	487928
Oligomycin	Inhibits membrane-bound mitochondrial ATPase.	495455
Paromomycin Sulfate	Aminoglycoside; inhibits bacterial protein synthesis. Also has anti-amoebic activity.	512731
Penicillin G (Benzylpenicillin)	Anti-bacterial. Inhibits bacterial cell wall synthesis.	5161
Polymyxin B Sulfate (Aerosporin)	Anti-bacterial. Disrupts the structure of the bacterial cell wall, causing leakage of intracellular compounds.	5291
Ribostamycin Sulfate	Aminoglycoside; inhibits bacterial protein synthesis.	557205
Rifampicin (Rifampin)	Anti-bacterial. Specifically inhibits DNA-dependent bacterial RNA polymerase, thus inhibiting RNA.	557303
Salinomycin	Anti-parasitic. Potassium-sensitive ionophore.	563080
Sisomicin	Aminoglycoside; inhibits bacterial protein synthesis.	567205
Spectinomycin	Aminoglycoside; inhibits bacterial protein synthesis.	567570
Streptomycin Sulfate	Aminoglycoside; inhibits bacterial protein synthesis.	5711
Streptozotocin	Anti-neoplastic and anti-bacterial. Causes DNA alkylation.	572201
Tobramycin	Aminoglycoside; inhibits bacterial protein synthesis. Also inhibits myeloperoxidase-dependent cell injury.	614005
Tunicamycin	Antibacterial, anti-fungal, and antiviral agent. Inhibits N-linked glycosylation and blocks formation of N-glycosidic protein carbohydrate linkages.	654380
Valinomycin	Potassium ionophore. Uncouples oxidative phosphylation.	676377
Vancomycin	Antibacterial. Inhibits bacterial cell wall synthesis.	627850

Reagents for Cell Selection

Product	Cat. No.	M.W.	Description	Stock Solution[a]	Suggested Conc. For Selection[b]	Refs.
Adenine	1152	135.1	Hypoxanthine substitute in XGPRT selection medium. Serves as a precursor for AMP via purine salvage when *de novo* synthesis is blocked by aminopterin.	0.5 mg/ml H_2O	XGPRT 25 µg/ml (185 µM)	21
5-Bromo-2´-deoxyuridine (5-BrdU)	203806	307.1	Thymidine analog used to select for loss of viral or cellular TK genes. Kills TK^+ cells as a result of its phosphorylation by TK and subsequent incorporation into DNA. Also commonly used to label DNA.	5 mg/mlm in DMSO or 100 mM NaOH	**TK^- selection:** 25-30 µg/ml	1, 9, 22, 25, 26
Hypoxanthine	4010	136.1	Ingredient of HAT, HMT, and AzaH hybridoma selection media and selection medium for the *E. coli gpt* (XGPRT) gene. Serves as a precursor for IMP by the purine salvage pathway when *de novo* synthesis is blocked by aminopterin, methotrexate, or azaserine.	**100X for HAT or HMT media:** In H_2O: 10 mM Hypoxanthine, 1.6 mM Thymidine; may require heating to 70°C. **For 100X AzaH medium:** 10 mM Hypoxanthine **670X for XGPRT medium:** 10 mg/ml in H_2O or 0.1 N NaOH	**HAT or HMT:** 100 µM (13.6 µg/ml) **AzaH:** 100 µM (13.6 µg/ml) **XGPRT:** 15 µg/ml (110 µM)	21-24, 27
Methotrexate, Disodium Salt (MTX)	454125	498.4	Dihydrofolate analog that inhibits DHFR, blocking *de novo* purine and pyrimidine nucleotide synthesis and forcing utilization of salvage pathways. Used to select for MTX^r conferred by a mutant DHFR gene or an amplified normal DHFR gene. Also used in HMT hybridoma selection medium.	Supplied at 25 ml (55 mM) in sterile isotonic NaCl solution. Protect from light. May dilute further before use: **For MTX^r:** 5 mM in H_2O/media. **100X for HMT medium:** 1 mM in H_2O	**For MTX^r:** 0.01-300 µM **HMT:** 10 µM	1,9, 23,28
Mycophenolic Acid	475913	320.4	Principle ingredient of XGPRT selection medium. Blocks conversion of IMP to XMP, the immediate precursor to GMP. Xanthine circumvents this block, as it is converted to XMP by XGPRT.	**400X for XGPRT medium:** 10 mg/ml in 0.1 N NaOH	**XGPRT:** 25 µg/ml (78 µM)	1,9, 23, 28
Ouabain, Octahydrate	4995	728.8	Glycoside that inhibits cell membrane Na^+,K^+-ATPase. Used for human hybridoma selection. Selection of human-mouse hybridomas exploits the greater sensitivity of human cells to ouabain and the sensitivity of mouse myeloma cells to HAT medium. Generation of human-human hybridomas requires the preselection of one cell line to Oua^r and HAT^s.	10 mg/ml (13.7 mM) in H_2O	0.1 - 10 µM	29-32
Thymidine	6060	242.2	Ingredient of HAT, HMT, and XGPRT selection media. Serves as a precursor for TMP by the pyrimidine slavage pathway when *de novo* synthesis is blocked by aminopterin or methotrexate.	**100X for HAT or HMT medium:** See hypoxanthine (Cat. No. 4010) **100X for XGPRT medium:** 1 mg/ml in H_2O	**HAT or HMT:** 16 µM (3.8 µg/ml) **XGPRT:** 41.3 µM (10 µg/ml)	21-23
Xanthine	6820	152.1	Ingredient of XGPRT selection medium. Utilized by XGPRT as a precursor of XMP, enabling cell growth in the presence of mycophenolic acid, a blocker of endogenous XMP (and thus GMP) production.	**40X for XGPRT medium:** 10 mg/ml in 0.1 N NaOH	**XGPRT:** 250 µg/ml (1.6 mM)	9, 21, 27

Notes on Usage:

a Stock solutions of drugs (except those in DMSO or 100% EtOH) may be sterilized by filtration through a 0.22 µm pore size filter. Solutions should be stored frozen (-20°C) in aliquots, with the exception of G 418 (all solutions), Hygromycin B (all solutions), and Methotrexate (25 mg/ml stock), which should be stored refrigerated (+4°C).

b The optimal concentrations of inhibitors and supplements necessary for selection varies with cell type, especially for cultured mammalian cells, and should be determined empirically by titration.

Abbreviations Used:

AMP = Adenosine 5´-monophosphate
GMP = Guanosine 5´-monophosphate
TMP = Thymidine 5´-monophosphate
UMP = Uridine 5´-monophosphate
IMP = Inosine 5´-monophosphate
XMP = Xanthine 5´-monophosphate
NMP = Nucleoside 5´-monophosphate

DHFR = Dihydrofolate reductase
TK = Thymidine kinase
APRT = Adenosine phosphoribosyltransferase
HGPRT = Hypoxanthine phosphoribosyltransferase
XGPRT = Xanthine-guanine phosphoribosyltransferase
FH2 = Dihydrofolate
FH4 = Tetrahydrofolate

HAT = Hypoxanthine/Aminopterin/Thymidine
HMT = Hypoxanthine/Methotrexate/Thymidine
AzaH = Azaserine/Hypoxanthine
Oua = Ouabain
MTX = Methotrexate
5-BrdU = 5-Bromo-2´-deoxyuridine
DMSO = Dimethyl sulfoxide
EtOH = Ethanol (ethyl alcohol)

References:

1. Maniatis, T., et al. 1989. *Molecular Cloning, A Laboratory Manual,* Second Edition. Cold Spring Harbor, New York.
2. Lech, K., and Brent, R. 1995. In: *Current Protocols in Molecular Biology,* Vol. 1 (Ausubel, F.M., et al., eds.) John Wiley & Sons, New York.
3. Kojima, N., et al. 1994. *J. Biol. Chem.* **269,** 30451.
4. Kimura, M., et al. 1994. *Biochem. Biophys. Acta* **1219,** 653.
5. Izumi, M., et al. 1991. *Exp. Cell Res.* **197,** 229.
6. Gatignol, A., et al. 1988. *FEBS Lett.* **230,** 171.
7. Mulsant, P., et al. 1988. *Somat. Cell Mol. Genet.* **14,** 243.
8. Ehrenfeld, G.M., et al. 1987. *Biochemistry* **26,** 931.
9. Kingston, R.E. 1995. In: *Current Protocols in Molecular Biology,* Vol. 1 (Ausubel, F.M., et al., eds.) John Wiley & Sons, New York.
10. Southern, P. J., and Berg, P. 1982. *J. Mol. Appl. Gen.* **1,** 327.
11. Santerre, R.F., et al. 1991. *Methods Mol. Biol.* **7,** 245.
12. Rees, W.D., et al. 1994. *Biochem J.* **299,** 637.
13. Giordano, T.J., and McAllister, W.T. 1990. *Gene* **88,** 285.
14. de la Luna, S., and Ortín, J. 1992. *Methods Enzymol.* **216,** 376.
15. Hechler, U., et al. 1993. *Biochem. Biophys. Res. Commun.* **194,** 1305.
16. Smokvina, T., et al. 1990. *Gene* **94,** 53.
17. Blanco, G., et al. 1992. *Gene* **112,** 59.
18. Hardman, J.G. 1996. *Goodman and Gilman's The Pharmacological Basis of Therapeutics,* Ninth Edition. McGraw-Hill Companies, New York.
19. Chopra, I. 1998. *J. Antimicrob. Chemother.* **41,** 584.
20. Sharma, R.C., and Schimke, R.T. 1994. *Mutat. Res.* **304,** 243.
21. Mulligan, R.C., and Berg, P. 1981. *Proc. Natl. Acad. Sci. USA* **78,** 2072.
22. Littlefield, J.W. 1964. *Science* **145,** 709.
23. Harlow, E., and Lane, D. 1988. *Antibodies: A Laboratory Manual.* Cold Spring Harbor, New York.
24. Jantscheff, P., et al. 1993. *J. Immunol. Methods* **163,** 91.
25. Earl, P.L., et al. 1995. In: *Current Protocols in Molecular Biology,* Vol.1 (Ausubel, F.M., et al., eds.) John Wiley & Sons, New York.
26. Mackett, M., et al. 1982. *Proc. Natl. Acad. Sci. USA* **79,** 7415.
27. Falkner, F.G., and Moss, B. 1988. *J. Virol.* **62,** 1849.
28. Simonsen, C.C., and Levinson, A.D. 1983. *Proc. Natl. Acad. Sci. USA* **80,** 2495.
29. Kozbor, D., et al. 1982. *Hybridoma* **1,** 323.
30. Kozbor, D., et al. 1982. *Proc. Natl. Acad. Sci. USA* **79,** 6651.
31. Lakow, E., et al. 1983. *J. Immunol.* **130,** 169.
32. Teng, N.N., et al. 1983. *Proc. Natl. Acad. Sci. USA* **80,** 7308.

Please call our Technical Service Department or your local sales office for more information on these products.

United Kingdom
Tel (0115) 943 0840
Fax (0115) 943 0951
customer.service@cnuk.co.uk

USA, Canada, & Mexico
Tel (800) 854-3417
Fax (800) 776-0999
technical@calbiochem.com

VWR Scientific Products
www.vwrsp.com
VWR CanLab
www.vwrcanlab.com
Tel (800) 932-5000

Germany
Tel (06196) 63955
Fax (06196) 62361
customer.service@calbiochem-novabiochem.de

For additional information, contact your local Calbiochem-Novabiochem sales office.

CB0613-0300