Biochrom AG's antibiotics solutions: Up-to-date overview regarding mechanism of action, performance and working concentration

Biochrom AG Information, November 19, 2010

Cell culture media allow not only cells but also foreign germs, such as bacteria or fungi, to grow ideally. This growth of foreign organisms, which is also referred to as "contamination", results nearly always in the loss of the respective cell culture. Only the preventive use of antibiotics or sterile working conditions may prevent contaminations. Originally, antibiotics (Greek "anti": against; "biotikos": relating to living organisms) was the term for low-molecular metabolites of microorganisms that act as growth inhibitors against other microorganisms or kill the latter. Today, the term antibiotics comprises all substances (also virostatics and chemotherapeutics) that are effective against any type of microorganism (bacteria, viruses, fungi), no matter if they are of low or high molecular nature or if they were produced synthetically or by the microorganisms themselves.

Antibiotics may be distinguished according to their effectiveness, chemical structure or their action mechanism. Within the framework of cell culture, it is rather effectiveness that is of importance: on the one hand, there are bacteriostatic antibiotics that only inhibit the growth of microorganisms, but do not kill them. On the other hand, there are bactericidal antibiotics, which not only inhibit growth, but additionally kill microorganisms.

Penicillin and Streptomycin are those two antibiotics that are used in cell culture on a routine basis. The cocktail referred to as "Pen/Strep" is effective against Gram-negative and Gram-positive bacteria, as well as against mycobacteria. Both antibiotics remain, however, stable in the culture for only a period of three days. If the cell culture is to be kept for a longer period of time, Pen/Strep has to be added again or replaced by more stable antibiotics. As Biochrom AG offers different types of antibiotics, the following overview covers some important criteria, such as working concentration, stability and toxicity, in accordance with latest research findings. The last part includes a short FAQ section regarding "antibiotics".

1 Classification of antibiotics

Antibiotics can be classified according to the following criteria:

- chemical structure
  β-lactam, aminoglycosides, macrolides, polypeptides, tetracyclines etc.

- effectiveness
  1. bacteriostatic antibiotics (inhibit growth of microorganisms, but do not kill them)
  2. bactericidal antibiotics (inhibit growth of microorganisms and kill them)
  In the case of bactericidal antibiotics, one may further distinguish between primary and secondary bactericides. Primary bactericides are effective against non-proliferating bacteria, while secondary bactericides are effective only against dividing bacteria.

- mechanism
  1. inhibition of cell wall synthesis: e.g. β-lactam, such as Penicillin and glycopeptides

2. inhibition of protein biosynthesis: e.g. aminoglycosides, tetracyclines, macrolides and others. A number of Biochrom antibiotics belong to the group of aminoglycosides, such as Gentamycin sulfate, Kanamycin, Neomycin, and Streptomycin.
3. disturbance of cell membrane function: e.g. polyene macrolides, such as amphotericin B, and peptides, such as Polymyxin B-sulfate.
4. interference with bacterial DNA or RNA: e.g. rifampicin.

2 Details on the individual antibiotics offered by Biochrom AG

table 1 and 2: details on the antibiotics Amphotericin B and Bacitracin

Parameter	Amphotericin B	
cat. no.	A 2612	A 2610 (lyophilised)
unit	50 ml (250 µg/ml)	1x (6x5 ml)
molecular formula	$C_{47}H_{73}NO_{17}$	
CAS no.	1397-89-3	
molecular weight	924.1 g/mol	
classification	polyene macrolide, antimycotic	
effects	fungi and yeasts, no effects on bacteria	
working concentration	2.5-3 µg/ml	
maximum solubility	40 mg/ml in DMSO, insoluble in H_2O, soluble in H_2O with Na-deoxycholate, storage at -20 °C	
stability[1]	3 days at 37 °C in solution	
toxicity[1]	30 µg/ml	
mechanism	Disturbs cell membrane permeability by binding to cell membrane sterols of fungi. Effective against, inter alia, *Candida* types. By binding to planar sterols, such as cholesterol, it may modify the cell membrane, which becomes permeable to ions (K^+, Mg^{2+})[2] and other low-molecular substances (e.g. amino acids, sugar, nucleotides)[3].	
isolated from	*Streptomyces nodosus*	

Parameter	Bacitracin
cat. no.	Biochrom AG offers the combination Bacitracin/Neomycin (see Neomycin)
molecular formula	$C_{66}H_{103}N_{17}O_{16}S$
CAS no.	1405-87-4
molecular weight	1422.72 g/mol (Bacitracin A), mixture of 9 Bacitracins
classification	polypeptide
effects	mainly on Gram-positive bacteria, but also on Mycoplasma and Neisseria
working concentration	0.1-1 mg/ml
maximum solubility	100 mg/ml in H_2O or ethanol, storage at -20 °C
stability[1]	quickly inactive at 25 °C in a pH range of > 5
mechanism	Inhibits cell wall synthesis of Gram-positive bacteria by binding to bactoprenyl pyrophosphate[4].
isolated from	*Bacillus licheniformis*

[1] Lindl, T. and Bauer, J. (2008): *Zell- und Gewebekultur*, 6th edition, Spektrum Akademischer Verlag, Heidelberg.
[2] Ellis, D. (2002): *Amphotericin B: spectrum and resistance*, J. Antimicrob Chem 49: 7-10.
[3] Holz, R. W. (1979): *Antibiotics V*, 313 ff, F. E. Hahn ed, Sringer Verlag Berlin, Heidelberg, New York.
[4] Scogin, D. A. *et al.* (1980): *Binding of nickel and zinc ions to bacitracin A*, Biochemistry 19 (14): 3348-52.

table 3 and 4: details on the antibiotics G 418-BC, Gentamycin-sulfate (Gentamicin)

Parameter	G 418-BC	
cat. no.	A 291-25	A 2912 (liquid, ready for use)
unit	10 g	50 ml
molecular formula	$C_{20}H_{40}N_4O_{10}$-$2H_2SO_4$	
CAS no.	108321-42-2	
molecular weight	692.7 g/mol	
classification	aminoglycoside	
effects	bacteria, fungi, yeasts, protozoa, and mammalian cells[5] [6], suited for the selection of transfected eukaryotic cells	
working concentration	50-1000 µg/ml	
maximum solubility	10-50 mg/ml in buffers or culture medium, storage at -20 °C	
stability	stable in solution at 2-8 °C for 6 months	
mechanism	Inhibits protein synthesis by disturbing the ribosome function.	
isolated from	*Micromonospora rhodorangea*	

Parameter	Gentamycin-sulfate (Gentamicin)				
cat. no.	A 2710 (lyophilised)	A 2712	A 271-23	A 271-25	A 271-26
unit	1x (6x5 ml)	50 ml	2.5 g	10 g	25 g
molecular formula	$C_{19\text{-}21}H_{39\text{-}43}N_5O_7$ x 2,5 H_2SO_4				
CAS no.	1405-41-0				
molecular weight	694.75-723.75 g/mol; complex of three chemically similar gentamicins: C_1, C_2 and C_{1a}[7]				
classification	aminoglycoside				
effects	Gram-positive and Gram-negative bacteria and Mycoplasma[8]				
working concentration	15-50 µg/ml				
maximum solubility	10-250 mg/ml in H_2O, storage at -20 °C				
stability[1]	5 days at 37 °C				
toxicity[1]	3 mg/ml				
mechanism	Inhibits protein biosynthesis by binding to the 30 S ribosomal subunit and by destroying the cell membrane[9].				
isolated from	*Micromonospora purpurea*				

[5] Waitz, J. A. *et al.* (1974): *Biological Activity of Antibiotic G-418, a New Micromonospora-Produced Aminoglycoside with Activity Against Protozoa and Helminths*, Antimicrob Agents Chemother 6 (5): 579-581.
[6] Panchal, C.J *et al.* (1984): *Susceptibility of Saccharomyces spp. and Schwanniomyces spp. to the Aminoglycoside Antibiotic G418*, AEM 47 (5): 1164-1166.
[7] Weinstein, M. J *et al.* (1967): *Biological Activity of the Antibiotic Components of the Gentamicin Complex*, J Bact 94 (3): 789-790.
[8] Rudin, A. *et al.* (1970): *Antibacterial Activity of Gentamicin Sulfate in Tissue Culture*, Appl Microbiology 20 (6): 989-990.
[9] Kadurugamuwa, J. *et al.* (1993): *Surface Action of Gentamicin on Pseudomonas aeruginosa*, J Bact 175 (18): 5798-5805.

table 5, 6 and 7: Kanamycin, Neomycin and Partricin

Parameter	Kanamycin
cat. no.	A 2512
unit	50 ml (5 mg/ml)
molecular formula	$C_{18}H_{36}N_4O_{11}$ x H_2SO_4
CAS no.	25389-94-0
molecular weight	582.58 g/mol, mixture of Kanamycin A and < 5 % Kanamycin B
classification	aminoglycoside
effects	Gram-positive and Gram-negative bacteria, as well as Mycoplasma
working concentration	50-100 µg/ml
maximum solubility	10 mg/ml in H_2O
stability[1]	5 days at 37 °C
toxicity[1]	10 mg/ml
mechanism	Binds to the 30 S ribosomal subunit, thus inhibiting protein biosynthesis[10].
isolated from	*Streptomyces kanamyceticus*

Parameter	Neomycin
cat. no.	A 2412 (combination Neomycin/Bacitracin)
unit	50 ml
molecular formula	$C_{23}H_{46}N_6O_{13}$ x $3H_2SO_4$
CAS no.	1405-10-3
molecular weight	908.88 g/mol, mixture of Neomycin B and C
classification	aminoglycoside
effects	Gram-positive and Gram-negative bacteria
working concentration	50 µg/ml
maximum solubility	100 mg/ml in H_2O or methanol, storage at -20 °C
stability[1]	at 37 °C, 5 days
toxicity	3 mg/ml
mechanism	Inhibits protein biosynthesis, inhibits phospholipase C and D[11]
isolated from	*Streptomyces fradiae*

Parameter	Partricin
cat. no.	A 2812
unit	50 ml
molecular formula	$C_{79}H_{119}O_{31}N_5$
CAS no.	-
molecular weight	1634.8 g/mol
classification	polyene antimycotic
effects	fungi and yeasts[12]
working concentration	0,5 µg/ml
maximum solubility	50 µg/ml in H_2O
stability[1]	3 days at 37 °C
mechanism	Inhibits proliferation
isolated from	*Streptomyces aureofaciens*

[10] Masukawa, H. (1969): *Localization of Sensitivity to Kanamycin and Streptomycin in 30S Ribosomal Proteins of Escherichia Coli*, J Antibiotics 22 (12):612-623.
[11] Liscovitch, M. *et al.* (1991): *Inhibition of neural phospholipase D activity by aminoglycoside antibiotics*, Biochem J 279: 319-321.
[12] Rimaroli C. and Bruzzese T. (1998): *In Vitro Activity of a New Polyene, SPA-S-843, against yeast*, Antimicrob Agents Chemother 42 (11): 3012-3013.

table 8, 9 and 10: details on Penicillin, Polymyxin B-sulfate and Streptomycin-sulfate; Pen/Strep

Parameter	Penicillin	
cat. no.	A 321-42	A 321-44
unit	25 mil. U	100 mil. U
molecular formula	$C_{16}H_{17}N_2NaO_4S$	
CAS no.	69-57-8	
molecular weight	356.4 g/mol	
classification	β-lactam	
effects	Gram-positive bacteria	
working concentration	20-100 µg/ml or 100 U/ml	
maximum solubility	10-100 mg/ml in H_2O	
stability[1]	at 37 °C , 3 days	
toxicity[1]	10 mg/ml	
mechanism	Inhibits the last cell wall synthesis, connects peptidoglycan strands by irreversibly interacting with transpeptidase[13]	
isolated from	*Penicillium chrysogenum* (previously known as *Penicillium notatum*)	

Parameter	Polymyxin B-sulfate
cat. no.	A 231-40
unit	5 mil. U
molecular formula	$C_{55}H_{96}N_{16}O_{13}$ x $2H_2SO_4$
CAS no.	1405-20-5
molecular weight	1385.63 g/mol
classification	polypeptide
effects	only Gram-negative bacteria, also against non-proliferating bacteria
working concentration	5-500 µg/ml
maximum solubility	10-20 mg/ml in H_2O or methanol, storage at -20 °C
stability[1]	5 days at 37 °C
toxicity[1]	3 mg/ml
mechanism	Binding to lipopolysaccharides of the Gram-negative bacteria leads to a permeabilisation of the cell membrane[14]. This results in loss of ions (Fe^{2+}, Mn^{2+}, Ca^{2+}, Mg^{2+}), unsaturated fatty acids and polyphosphate.
isolated from	*Bacillus polymyxa*

Parameter	Streptomycin-sulfate		
cat. no.	A 331-26	A 331-27	
unit	25 g	100 g	
molecular formula	$(C_{21}H_{39}N_7O_{12})_2$-$3H_2SO_4$		
CAS no.	3810-74-0		
molecular weight	1457.4 g/mol		
classification	aminoglycoside		
effects	primarily Gram-negative bacteria and Mycobacteria		
working concentration	10-100 µg/ml		
maximum solubility	10-50 mg/ml in H_2O		
stability[1]	3 days at 37 °C		
toxicity[1]	20 mg/ml		
mechanism	Inhibits initiation of the protein biosynthesis[15]		
isolated from	*Streptomyces griseus*		
combination	**Pen/Strep**		
cat. no.	A 2210 (lyophilised)	A 2212 (liquid)	A 2213 (liquid)
unit	1x (6x5 ml)	50 ml	100 ml

[13] Izaki, K. *et al.* (1966): *Glycopeptide Transpeptidase and D-Alanine Carboxypeptidase: Penicillin-Sensitive Enzymatic Reactions*, Proc Natl Acad Sci 55 (3): 656-663.
[14] Koike, M. *et al.* (1968): *Electron Microscopic Studies on Mode of Action of Polymyxin*, J Bact 97: 448-452.
[15] Luzzatto, L. *et al.* (1968): *Mechanism of Action of Streptomycin in E.coli: Interruption of the Ribosome Cycle at the Initiation of Protein Synthesis*, Proc Natl Acad Sci 60 (3): 873–880.

3 Frequently asked questions about antibiotics

3.1 How do I notice a contamination?

The medium turns cloudy. If the medium contains phenol red as pH indicator, the colour of the medium changes from red to yellow-orange in most cases, as the metabolites of the microorganisms accumulate, resulting in a change of the pH-value. Contaminated cultures may also be identified by a variation of the odour. The use of a microscope ensures a very quick identification of microorganisms (except for mycoplasma).

3.2 The culture has been contaminated despite preventive treatment with antibiotics. What may be the reasons?

There are several reasons:

a. The dose of the antibiotic in the medium is too low. The individual working concentration of the antibiotic in the medium differs (see tables above) and should be maintained as precisely as possible.

b. In addition, antibiotics in the medium, depending on the respective antibiotic, remain stable at 37 °C for approx. 3 to 5 days. At refrigeration temperatures between 2 and 8 °C, antibiotics show more stability. Culture medium that has been supplemented by antibiotics should, however, not be kept in a refrigerator too long.
For example, if a culture is to grow over a period of five days, an appropriately stable antibiotic needs to be used. Pen/Strep remains stable for only three days. Due to its spectrum, Kanamycin (see tables above) may represent an alternative to Pen/Strep, as it remains stable for five days.

c. The antibiotic used is not effective because the microorganisms have mutated and are now resistant against the antibiotic (to continue see point 3.4).

3.3 What happens if the antibiotics have been dosed too high?

Antibiotics that have been dosed too high may be toxic. As a result, cells die off. Working concentration values for the individual antibiotics substances are to be exactly adhered.

3.4 May the microorganisms become resistant during treatment?

During treatment, microorganisms may mutate and become resistant against the antibiotic used. You may prevent such resistance in the case of long-term cultures by stopping the use of antibiotics completely for two or three passages.

3.5 May antibiotics successfully eliminate Mycoplasma?

Mycoplasma considerably impairs cell proliferation through nutrient competition and cytotoxic secretion. Test-positive cultures often have to be rejected, as treatments with antibiotics are usually too complex or ineffective.

In order to eliminate contaminations caused by Mycoplasma, antibiotics are used that feature high stability in the culture medium and are less toxic at low concentrations, such as ~~Kanamycin (cat. no. A 2512). To do so, the culture medium is supplemented with 100 µg/ml~~

Kanamycin (cat. no. A 2512). To do so, the culture medium is supplemented with 100 µg/ml Kanamycin.

Our recommendation: As an effective alternative to antibiotics, Biochrom AG recommends the Mynox® elimination reagent, which immediately kills Mycoplasma and acts on a biophysical basis (for more information go to http://www.biochrom.de/en/products/mycoplasma-reagents/.)

3.6 How may I check if the treatment was successful?

Cultivate the cell culture for one or two passages without adding antibiotics.