

# Bac-Off®

## Cat# NB-11-0074

### Introduction

Bac-Off® Tonic for antibiotic treatment of media.

This product was developed to address the problem of unpredictable, inevitable bacteria in cell culture. Cells have, as a natural and normal part of their constitutive programming, the ability to enclose bacteria adherent to their lumenal surface in membrane, and sink them into the cytoplasm. The bacteria, while in these vacuoles do not divide, except if already triggered into the cell cycle, and the resulting doublets do not divide (at least for times on the scale of hours). If the bugs are released, they proliferate briskly. At high multiplicities, cells containing bac-vacuoles are adversely affected, exhibit toxicity and measurable loss of specific activity. The eventual release of even a single viable bacteria from sequestration has predictable and dismal consequences. This product is formulated at MIC to inhibit the growth of most bacterial agents and control contamination in cell culture, without harming the cells.

Bac-Off® is intended to provide practical relief to the scientist by exposing the bacteria to effective oblivion from the point of view of the experiment by placing it, once released, in a medium environment containing the adduced MIC of a quinolone antibiotic, acting to interfere with the chromosomal breakage-reunion reaction of bacterial gyrase and human topoisomerase II. Bacterial replication is coupled with and absolutely dependant on chromosomal replication, with as many as several waves of chromosomal replication present in log cultures. In the presence of Bac-Off® at MIC dosage, bugs are unable to relax replication fork driven supercoiling and are kicked into a prolonged G2 shunt (which could eventually, be fatal). As a practical matter, bacteria released into the medium will be washed away during routine feeding operations.

Dosage of Bac-Off® was established by identifying the MIC for the bug and the toxicity dosage for human microvascular cells. Nearly twenty-five individual isolates of ACBRI Primary Human Microvascular cells have been probed to date. Results are emerging that indicate that the effective MIC is  $2\mu g/mL$  (mcg/mL in the parlance of the clinical lab) and that visible toxicity is not encountered until one is north of  $\sim 10\mu g/mL$ .

### **Available Formats**

Comes in 4mL (NB-11-0074) size



## **Appropriate Use**

Cells are at their most vulnerable when in recovery from thawing or passage. Add 1mL of Bac-Off® to each 500mL of media. Feed culture, as usual. If you aliquot and freeze your medium, add Bac-Off® before dividing and freezing media.

## **Handling and Storage**

Store Bac-Off® at -20°C until ready to use. If an entire 500 mL unit of CSC media will not be used within 30 days, activate the medium with growth supplement and Bac-Off then aliquot and freeze in smaller units which will be used within 30 days (store at +4 - 8°C)

CSC media and reagents are Sterile, made with WFI and all components are cGMP and ISO Compliant.

## **Additional Information**

Bac-Off® is delivered as a 500X concentrate which, at working strength (1:500 v/v dilution in complete medium) exhibits Minimum Inhibitory Concentration (MIC) according to standard protocols against the listed species of microorganism.

Bac-Off® may also be used at double working strength (1:250 v/v) at which concentration it exhibits Minimum Bactericidal Concentration (MBC), which generally does not exceed the MIN by more than a factor of 2.

Bac-Off® may also be used at ten times working strength (1:50 v/v) at which concentration it exhibits mycoplasmacidal properties. This dosage is well tolerated by most mammalian cells in vitro for short periods of time.

Bac-Off® contains the synthetic fluoroquinone Ciprofloxacin, with a wide range of in vitro activity against a wide range of gram-negative and gram-positive microorganisms, specifically including the following:

Active against the following microorganisms:

Bacillus fragilis Clostridium difficile Staphylococcus spp

Staphylococcus haemolyticus Staphylococcus epidermidis

Staphylococcus saprophyticus Staphylococcus hominis

Streptococcus pneumoniae Streptococcus pyogenes

Pseudomonas aeruginosa Haemophilus influenzae

Haemophilus parainfluenzae Enterococcus faecalis Escherichia coli

Enterobacter spp. Enterobacter cloacae



Enterobacter aerogenes Citrobacter spp. Citrobacter diversus

Citrobacter freundii Klebsiella spp. Klebsiella pneumoniae

Klebsiella oxytoca Moraxella catarrhalis Morganella morganii

Proteus spp. Proteus mirabilis Proteus vulgaris

Providencia Spp. Providencia stuartii Providencia rettgeri

Serratia marcescens Acinetobacter Lwoffii Aeromonas hydrophilia

Campyhlobacter jejuni Edwardsiella tarda Legionella pneumophilia

Neisseria gonorrhoeae Salmonella spp. Salmonella enteritidis

Salmonella typhii Shigella spp. Shigella dysenteriae

Shigella boydii Shigella flexneri Shigella sonnei

Vibrio spp. Vibrio Cholerae Vibrio vulnificus

Yersinia spp. Yersinia enterocolitica Yersinia pestis

#### Bacillus anthracis

The mechanism action of Bac-Off® is distinct from that of penicillins, cephaslosporins, aminoglycosides, macrolides, and tetracyclines. There are no reported instanxces of cross-resistance between Bac-Off® and other classes of antimicrobials. The inoculum size has little effect when tested in vitro.

The bacteriastastic ("working strength" 1:500) and bacteriacidal ("double strength" 1:250) activity of Bac-Off® results from inhibition of the bacterial enzymes topoisomerase II (DNA gyrase) and topoisomerase IV. These enzymes are necessary for bacterial replication, transcription, and repair. Mammalian topoisomerases are not affected by Bac-Off® at less than 7-10 fold working strength. This is a much wider "window" between the dosage active against bacterial growth and the dosage toxic to mammalian cells than all other commonly used antibiotic classes.

No application of sterile technique is 100% foolproof. Bac-Off® at working strength (MIC) is not acting as an antibiotic per se. Operationally it is, simply, a virtual certainty that no bacterial contamination will occur while Bac-Off® is in use

Bac-Off® is stable for at least six months at refrigerator temperatures, and may be frozen (<-20C) one time without loss of activity. No special precautions are necessary for disposal of Bac-Off® solutions at working or double strength.