Campylobacter Selective Supplement (Butzler)

PRODUCT INFORMATION

B002-5g - Bacitracin USP, Powder, 5g

B002-25KU - Bacitracin USP, Powder, 25KU

B002-25g - Bacitracin USP, Powder, 25g

C001-1g - Cycloheximide, Powder, 1g

C001-5g - Cycloheximide, Powder, 5g

C039-100mg - Colistin Sulfate, Powder, 100mg

C039-1g - Colistin Sulfate, Powder, 1g

C006-100mg - Cefazolin Sodium, Powder, 100mg

C006-500mg - Cefazolin Sodium, Powder, 500mg

C006-1g - Cefazolin Sodium, Powder, 1g

DESCRIPTION

Columbia Blood Agar Base with Campylobacter Selective Supplement (Butzler) is a selective medium for the isolation of *Campylobacter* species at 35°C as well as 43°C.

BACKGROUND

Bacitracin is a mixture of related cyclic polypeptides produced by organisms of the licheniformis group of *Bacillus subtilis* var Tracy, isolation of which was first reported in 1945.

Cycloheximide is widely used in biomedical research to inhibit protein synthesis in eukaryotic cells studied in vitro (i.e. outside of organisms). Its effects are rapidly reversed by simply removing it from the culture medium.

Colistin is a polymyxin antibiotic produced by certain strains of *Bacillus polymyxa* var. *colistinus*. Colistin is a mixture of cyclic polypeptides colistin A and B. Colistin is effective against most Gram-negative bacilli and is used as a polypeptide antibiotic.

Cephazolin is a first-generation cephalosporin antibiotic.

Novobiocin is an aminocoumarin antibiotic that is produced by the actinomycete *Streptomyces niveus*, which has recently been identified as a subjective synonym for *S. spheroides* a member of the order Actinobacteria

Mechanism of action

Bacitracin interferes with the dephosphorylation of the C55-isoprenyl pyrophosphate, a molecule that carries the building-blocks of the peptidoglycan bacterial cell wall outside of the inner membrane.

Cycloheximide is an inhibitor of protein biosynthesis in eukaryotic organisms, produced by the bacterium Streptomyces griseus. Cycloheximide exerts its effect by interfering with the translocation step in protein synthesis (movement of two tRNA molecules and mRNA in relation to the ribosome) thus blocking translational elongation.

Colistin is polycationic and has both hydrophilic and lipophilic moieties. These poly-cationic regions interact with the bacterial outer membrane, by displacing bacterial counter ions in the lipopolysaccharide. Hydrophobic/hydrophillic regions interact with the cytoplasmic membrane just like a detergent, solubilizing the membrane in an aqueous environment. This effect is bactericidal even in an isosmolaric environment.

The molecular basis of action of novobiocin, and other related drugs clorobiocin and coumermycin A1 has been examined. Aminocoumarins are very potent inhibitors of bacterial DNA gyrase and work by targeting the GyrB subunit of the enzyme involved in energy transduction. Novobiocin as well as the other aminocoumarin antibiotics act as competitive inhibitors of the ATPase reaction catalysed by GyrB. The potency of novobiocin is considerably higher than that of the fluoroquinolones that also target DNA gyrase, but at a different site on the enzyme. The GyrA subunit is involved in the DNA nicking and ligation activity.

APPLICATION IN COLUMBIA BLOOD AGAR BASE

Campylobacter Selective Supplement (Butzler) is based on the formulation of Butzler. The antibiotic supplement is designed to be used at 35°C as well as 43°C for optimum selective effect.

Polymyxins bind to the cell membrane and alter its

structure, making it more permeable. The resulting water uptake leads to cell death.

Content concentrations

Typical Formula*	mg/litre
Columbia Blood Agar Base	
Special peptone	23
Starch	1
Sodium chloride	5
Agar	10
Final pH 7.3 ± 0.2 @ 25°C	
Campylobacter Selective Supplement (Butzler)	
Bacitracin	25,000 IU (≈ 338 mg)
Cycloheximide	50
Colistin sulphate	10,000 IU
Cephazolin sodium	15
Novobiocin	5
* Adjusted as required to meet performance standards	

Table 1 typical formula for Columbia Blood Agar Base and Campylobacter Selective Supplement (Butzler)

METHOD

Preparation

Add appropriate amount of Columbia blood agar base to distilled water. Boil to dissolve and sterilise by autoclaving at 121°C for 15 minutes. Aseptically add supplements reconstituted as directed. Mix gently and pour into sterile Petri dishes.

Protocol

1. Prepare Campylobacter Selective Supplement (Butzler) plates as described in the preparation for use.

2. Emulsify approximately 0.5 g of the specimen in 5 ml of sterile 0.1% peptone water to form a 1:10 dilution.

3. Inoculate on to selective medium with cotton tipped swabs so that single isolated colonies are formed.

4. Incubate the plates in an atmosphere consisting of approximately 5-6% oxygen, 10% carbon dioxide and 84-85% nitrogen for 48 hours at 42°C.

5. Examine the plates and confirm the typical colonies as *Campylobacter* species.

Quality control

Positive control:

Campylobacter jejuni ATCC® 33291: Good growth; grey brown coloured colonies

Negative control:

Escherichia coli ATCC® 25922: Inhibited

REFERENCES

1. Goossens, H., M. De Boeck, and J. Butzler, A new selective medium for the isolation ofCampylobacter jejuni from human faeces. European Journal of Clinical Microbiology & Infectious Diseases, 1983. 2(4): p. 389-393.