Protocol



TD-P Revision 2.0

Creation Date: 7/10/2014 Revision Date: 3/6/2019

Cefotaxime 100 mg/ml EZ-Pak™ Protocol

Introduction

Cefotaxime is a third generation, broad-spectrum cephalosporin antibiotic effective against various gram-negative and gram-positive bacteria. However, unlike many other cephalosporins, cefotaxime is not effective against *Pseudomonas aeruginosa* bacteria. Cefotaxime is often used in *Agrobacterium tumefaciens*-mediated transformations to regulate bacterial growth. It has been shown to exhibit a synergistic effect with Vancomycin in certain conditions. Cephalosporins are a type of β -lactam antibiotic consisting of a four-membered β -lactam ring bound to a six-membered dihydrothiazine ring. This two-ring system causes distortion of the β -lactam amide bond, resulting in decreased resonance stabilization and increased reactivity. β -lactams inhibit the formation of peptidoglycan cross-links within bacterial cell walls by targeting penicillin-binding proteins or PBPs. Consequently, the bacterial cell wall becomes weak and cytolysis occurs. Cephalosporins are less susceptible to β -lactamases than the penicillin β -lactam antibiotics.

The Cefotaxime (Sodium) EZ Pak^{TM} is the fastest and easiest way to make a set amount of sterile cefotaxime (sodium) solution. The kit includes pre-weighed cefotaxime (sodium) powder, a sterile filter and a sterile container for the filtered solution. No need to calculate, simply add the stated amount of deionized H_2O , filter, and pour into the labeled bottle for easy usage. The EZ Pak^{TM} includes high quality GoldBio cefotaxime (sodium) and the sterile solution is ready for tissue culture, bacterial media or any number of uses.

Materials

- 1 Bottle of Cefotaxime Sodium powder
- 1 Sterile empty bottle for solution
- 1 Sterile Filter (and syringe for EZ10)

Method

Reconstitution Protocol

- 1. Warm Cefotaxime powder bottle to Room Temperature
- 2. Add specified volume of dH₂O to Cefotaxime powder bottle.



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| Product Catalog # | Volume of H ₂ O to Add | Final Volume |
|--------------------|-----------------------------------|--------------|
| <u>C-104-EZ10</u> | 9.4 ml | 10 ml |
| <u>C-104-EZ50</u> | 47.0 ml | 50 ml |
| <u>C-104-EZ100</u> | 94.0 ml | 100 ml |

- 3. Mix until all product goes into solution.
- 4. Sterile Filter:
 - a. For C-104-EZ10
 - i. Remove syringe from packaging.
 - ii. Carefully remove top of sterile filter packaging.
 - iii. Suck up as much solution as possible into syringe.
 - iv. Screw the Leur end of the syringe into the top of the sterile filter.
 - v. Carefully place the filter assembly above the empty bottle, and slowly depress the syringe plunger. Allow all solution to flow through the filter.
 - vi. Once all solution has been filtered, close the top of the solution bottle and store at
 - -20°C. Make aliquots if desired. Discard filter and syringe.
 - b. For C-104-EZ50 and EZ100
 - i. Remove vacuum filter from packaging.
 - ii. Attach vacuum hose according to instructions on filter packaging.
 - iii. Add solution to the upper cup of the filter.
 - iv. Apply vacuum pressure and let all the solution in the top cup flow through the filter into the bottom cup. Stop vacuum when all solution is filtered.
 - v. Remove vacuum attachment from filter and close bottle with provided sterile cap.
 - vi. Tightly seal solution bottle and store at -20°C. Make aliquots if desired. Discard filter.
- 5. Use Cefotaxime at a final concentration of 100 μg/ml.