

## Carbenicillin 100 mg/ml EZ-Pak™ Protocol

### Introduction

Carbenicillin is a member of the penicillin  $\beta$ -lactam antibiotics. However, unlike most  $\beta$ -lactams, carbenicillin disodium is limited to primarily gram-negative bacteria including *Pseudomonas aeruginosa* and common enteric species. It can be used in selection protocols and is often preferred over ampicillin due to its increased stability. This allows for less contamination of unwanted bacterial colonies. In addition, carbenicillin has been used to regulate bacterial growth in plants, yielding low regeneration frequencies. It can be used in genetic transformation applications to select for *AmpR* transformed cells.

Penicillins are a type of  $\beta$ -lactam antibiotic consisting of a four-membered  $\beta$ -lactam ring bound to a five-membered thiazolidine ring. This two-ring system causes distortion of the  $\beta$ -lactam amide bond, resulting in decreased resonance stabilization and increased reactivity.  $\beta$ -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. Resistance to  $\beta$ -lactam antibiotics occurs in the presence of cells containing plasmid encoded extended spectrum  $\beta$ -lactamases or ESBLs.

The Carbenicillin (disodium) EZ Pak™ is the fastest and easiest way to make a set amount of sterile carbenicillin (disodium) solution. The kit includes pre-weighed carbenicillin (disodium) powder, a sterile filter, and a sterile container for the filtered solution. No need to calculate, simply add the stated amount of deionized H<sub>2</sub>O, filter, and pour into the labeled bottle for easy usage. The EZ Pak™ includes high quality GoldBio carbenicillin (disodium) and the sterile solution is ready for tissue culture, bacterial media, or any number of uses.

### Materials

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

### Method

#### Reconstitution

1. Warm Carbenicillin powder bottle to Room Temperature.
2. Add specified volume of dH<sub>2</sub>O to Carbenicillin powder bottle.

Product Catalog #	Volume of H <sub>2</sub> O to Add	Final Volume
<a href="#">C-103-EZ10</a>	9.4 ml	10 ml
<a href="#">C-103-EZ25</a>	23.5 ml	25 ml
<a href="#">C-103-EZ50</a>	47.0 ml	50 ml
<a href="#">C-103-EZ100</a>	94.0 ml	100 ml

3. Mix until all product goes into solution.
4. Sterile Filter:
  - a. For C-103-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-103-EZ25, 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 Carbenicillin at a final concentration of 50-100 µg/ml.