

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

### Introduction

Timentin™ is a 15:1 mixture of ticarcillin and clavulanate. Ticarcillin is a penicillin  $\beta$ -lactam antibiotic, which is susceptible to  $\beta$ -lactamase degradation. When combined with clavulanate, a  $\beta$ -lactamase inhibitor, its efficacy is greatly increased. Timentin™ exhibits high activity against gram-negative bacteria and resistant *Agrobacterium* species.

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 Timentin™ EZ Pak™ is the fastest and easiest way to make a set amount of sterile Timentin™ solution. The kit includes pre weighed Timentin™ 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 Timentin™ and the sterile solution is ready for tissue culture, bacterial media, or any number of uses.

### Materials

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

### Method

#### Reconstitution Protocol

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

Product Catalog #	Volume of H <sub>2</sub> O to Add	Final Volume
<a href="#">T-104-EZ10</a>	9.6 ml	10 ml

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<a href="#">T-104-EZ50</a>	48.0 ml	50 ml
<a href="#">T-104-EZ100</a>	96.0 ml	100 ml

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3. Mix until all product goes into solution.
  
4. Sterile Filter:
  - a. For T-104-EZ10-
    - i. Remove syringe from packaging.
    - ii. Carefully remove top of sterile filter packaging.
    - iii. Aspirate as much solution as possible into the 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 the 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 T-104-EZ50 and EZ100-
    - i. Remove vacuum filter from packaging.
    - ii. Attach the 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 the solution is filtered.
    - v. Remove the vacuum attachments from filter and close the bottle with the provided sterile cap.
    - vi. Tightly seal the solution bottle and store at -20°C. Make aliquots if desired. Discard filter.
  
5. Use Timentin™ at a final concentration of 100 µg/ml.