

## Hygromycin EZ-Pak™ Protocol

### Introduction

Hygromycin B is an aminoglycoside antibiotic isolated from *Streptomyces hygroscopicus*. It is commonly used to study protein synthesis. Aside from its efficacy against bacteria, fungi, and higher eukaryotic cells, hygromycin B is also an antiviral agent inhibiting translation upon selectively entering cells made permeable due to viral infection. It works as a standard selection antibiotic in gene experiments, especially for selection of hygromycin resistance gene transformed cells. Aminoglycoside antibiotics are composed of amino groups attached to glycosides. They bind the 30s ribosomal subunit, causing misreading of the mRNA sequence and inhibition of translocation. Consequently, protein synthesis is inhibited.

The Hygromycin B EZ Pak™ is the fastest and easiest way to make a set amount of sterile hygromycin B solution. The kit includes pre-weighed hygromycin B 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 hygromycin B and the sterile solution is ready for tissue culture, bacterial media or any other appropriate use.

The 25 ml, 50 ml, and 100 ml pack sizes require a vacuum manifold for easy vacuum filtering, while the 10 ml pack size comes with a syringe filter. The EZ Pak™ includes high quality GoldBio Hygromycin and the sterile solution is ready for tissue culture, bacterial media, or other appropriate use.

### Materials

- 1 Bottle of Hygromycin powder
- 1 Sterile empty bottle for solution
- 1 Sterile Filter

### Method

Reconstitution protocol.

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

Product Catalog #	Volume of H <sub>2</sub> O to Add	Final Volume
<a href="#">H-270-EZ25</a>	24.0 ml	25 ml
<a href="#">H-270-EZ250</a>	48.0 ml	50 ml
<a href="#">H-270-EZ100</a>	96.0 ml	100 ml

3. Mix until all product goes into solution.
4. Sterile Filter:
  - a. For H-270-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 Hygromycin at a final concentration between 100 µg/ml and 1000 µg/ml (typically 200 µg/ml, depending on cell line).