Protocol

Creation Date: 6/1/2016
Revision Date: 10/4/2018

Hygromycin B Cell Culture and Plate Preparation

For Bacteria, Plant and Mammalian Cells and Yeast

Introduction

Hygromycin B is an aminoglycoside antibiotic produced by *Streptomyces hygroscopicus* that inhibits translocation of ribosomes during translation elongation, resulting in protein synthesis inhibition. This antibiotic's ability to kill bacteria, fungi, and higher eukaryotes has been useful in biomedical and plant research because it functions as a selection marker. Cells that carry the 1 Kb *hph* gene produce a kinase that phosphorylates Hygromycin B and thus, are resistant and survive. Here, we describe a general protocol for the use of Hygromycin B in selection of bacteria, plants, mammalian cells and yeast.

Materials

- Hygromycin B (GoldBio Catalog # H-270[ES])
- Yeast extract
- Bacto-peptone
- Dextrose
- HCl 1M
- Bacto Agar
- Trypsin (GoldBio Catalog # T-160)
- Murashige and Skoog (MS) medium
- Regeneration medium
- Competent bacteria
- Yeast
- Calli

Stock Solution and Working Concentrations

- Use this protocol to prepare either a stock solution of 50 or 100 mg/ml Hygromycin.
- Dilute to the following concentrations, based on the application:
    - For bacteria selection: 20-200 µg/ml.
    - For plant cell maintenance: 20 µg/ml.
    - For plant cell selection: 20-200 µg/ml.
    - For mammalian cell maintenance: 200 µg/ml.
    - For mammalian cell selection: Ranges from 50-1000 µg/ml.
    - For yeast selection: 200 µg/ml.

E S: EZ-Pak and Solution available

Gold Biotechnology
St. Louis, MO
Ph: (800) 248-7609
Web: www.goldbio.com
Email: contactgoldbio86@goldbio.com

Method

Preparation of Yeast Extract-Peptone-Dextrose (YPD) liquid medium and plates with drugs

1. Mix yeast extract (10 g/L), Bacto peptone (20 g/L), and dextrose (20 g/L) in 1 L of (deionized or distilled) H_2O.

2. Adjust the pH to 5.5 with 1M HCl and autoclave.

Preparation of YPD plates with Hygromycin B

1. Prepare liquid medium as above, but add Bacto Agar (20 g/L) before autoclaving.

2. Add Hygromycin B at desired concentration (concentration varies and must be optimized).

3. Allow medium to cool to 55-60°C. then pour into sterile Petri dishes.

Selection of Yeast

1. Following transformation, incubate culture at 30°C for 2-3 hours before plating on YPD plates containing 200 µg/ml.

Selection of Mammalian Cells

1. Transfect cells, then incubate for 48 hours in medium without Hygromycin B.

2. Trypsinize cells and plate in a 1:3 dilution using medium without Hygromycin B.

3. Twenty-four hours after trypsinization, replace the antibiotic-free medium with medium containing the appropriate concentration of Hygromycin B.

   Note: Hygromycin concentration needs to be optimized according to cell line.

   Note: Replace medium containing Hygromycin B after 2-3 days.

4. Determine if foci are present after 7 days of selection and isolate them after 10 days in culture.

Selection of Plants

1. Perform transformation with a plasmid containing the selectable marker *hpt* conferring resistance against Hygromycin B.

2. Transfer calli 12 hours after transformation to MS medium and appropriate additives (depends on the type of plant) and place in dark conditions.

3. After 48 hours place the calli in Petri dishes with the same medium and Hygromycin B (at desired concentration).

4. Culture for 4-6 weeks with fortnight subculturing for somatic embryo development.

5. Transfer to regeneration medium without Hygromycin B.

Preparation of agar plates with Hygromycin B

1. Prepare LB media by adding LB agar powder to 500 ml of deionized water in a flask.

2. Autoclave the LB media in a liquid cycle.

3. Cool the LB media to ~60°C (minimum of 30 minutes).

4. Add Hygromycin B to desired concentration.

5. Mix well by swirling.

6. Pour about 10 ml into sterile plates. Let the media solidify at room temperature, overnight.

   Note: Do not allow formation of bubbles.

7. When cool and solidified, wrap the plates with aluminum foil, and store at 4°C.

Selection of Bacteria

1. Thaw competent bacteria on ice.

2. Add 100 µl of thawed bacteria and plasmid DNA into a sterile tube, incubate on ice for 30 minutes.

3. Heat the bacteria and DNA at 42°C for 45 seconds. Then immediately place on ice for 5 minutes.

4. Add 1 ml of LB broth and incubate on a shaker at 37°C for 2 hours.

   Note: At this point warm up LB agar plates with hygromycin (at desired concentration) by placing them in a 37°C incubator.

5. Spread 100 µl of the bacteria/DNA solution on LB agar plates

Associated Products

- Hygromycin B (GoldBio Catalog # H-270)
- Trypsin (GoldBio Catalog # T-160)

References

Cameron, A. and Gaynor, E. C. (2014). Hygromycin B and Apramycin Antibiotic Resistance Cassettes for Use in Campylobacter jejuni. *PLoS ONE, 9*(4). Doi:10.1371/journal.pone.0095084.

Krantz, B. (n.d.). Pouring agar plates. Retrieved May 16, 2018, from http://mcb.berkeley.edu/labs/krantz/protocols/agar_plates.pdf.

Macgregor, G. R., Mogg, A. E., Burke, J. F., and Caskey, C. T. (1987). Histochemical staining of clonal mammalian cell lines expressingE. coli beta galactosidase indicates heterogeneous expression of the bacterial gene. *Somatic Cell and Molecular Genetics, 13*(3), 253-265. Doi:10.1007/bf01535207.

Ortiz, J. P., Reggiardo, M. I., Ravizzini, R. A., Altabe, S. G., Cervigni, G. D., Spitteler, M. A., . . . Vallejos, R. H. (1996). Hygromycin resistance as an efficient selectable marker for wheat stable transformation. *Plant Cell Reports, 15*(12), 877-881. Doi:10.1007/bf00231579.

Plackett, A. R., Huang, L., Sanders, H. L., and Langdale, J. A. (2014). High-Efficiency Stable Transformation of the Model Fern Species Ceratopteris richardii via Microparticle Bombardment. *Plant Physiology, 165*(1), 3-14. Doi:10.1104/pp.113.231357.

Rao, R. N., Allen, N. E., Hobbs, J. N., Alborn, W. E., Kirst, H. A., and Paschal, J. W. (1983). Genetic and enzymatic basis of hygromycin B resistance in Escherichia coli. *Antimicrobial Agents and Chemotherapy, 24*(5), 689-695. Doi:10.1128/aac.24.5.689.

YPD Liquid Medium and Plates with Drugs. (2017). *Cold Spring Harbor Protocols,* (4). Doi:10.1101/pdb.rec089516.