Protocol

G418 Culture and Plate Preparation Protocol

For Bacteria, Plant and Mammalian Cells, and Yeast

Introduction

G418 (also known as Geneticin®) is an aminoglycoside antibiotic produced by *Micromonospora rhodorangea* and is similar to gentamicin B1. It acts by blocking polypeptide synthesis by inhibiting the elongation step in cells. This antibiotic is commonly used in laboratories to select genetically engineered cells such as KanMX, with G418 resistance conferred by aminoglycoside 3'-phosphotransferase (APT 3'II), which is encoded by the neo gene in the Tn5 transposon. In general, lower concentrations of G-418 are used for cell maintenance, while higher concentrations are used for cell selection. In this protocol, we outline the preparation and use of G418 in cultures and plates.

Materials

- G418 Sulfate (GoldBio Catalog # G-418)
- Modified Cells (Bacteria, Plant, etc.)
- Cell culture media
- Sterile water

Storage and Handling

- Stock solution should be stored at -20°C and is stable for 1 year.
- This product is shipped at room temperature and should be stored desiccated at -20°C immediately upon arrival.

Method

Stock Solution

1. Use this protocol to prepare a stock solution of 200 mg/ml G418.

Working Solutions

1. Dilute to the following concentrations, based on the desired applications:
    a. For bacteria selection: 5-16 µg/ml.
    b. For plant cell maintenance: 10 µg/ml.
    c. For plant cell selection: 10-50 µg/ml.
    d. For mammalian cell maintenance: 200 µg/ml.

e. For mammalian cell selection: 400 µg/ml, with an optional concentration range of 300-1000 µg/ml.
f. For yeast: following transformation, incubate culture at 30°C for 2-3 hours before plating on YPD containing 200 µg/ml.

Note: For information on developing the proper kill curve for your culture, see our protocol on developing a titration kill curve.

Note: It is important to develop a kill curve for cell selection. G418's suggested concentration varies between cell types and cell modifications.

Procedure for Bacteria and Plant Cell Selection

1. G418 will select neo-carrying cells at concentrations as low as 5 µg/ml.

Note: It is advised to carefully develop the kill curve as increase in concentration can lead to sudden decrease in cell numbers across the entire culture.

Procedure for Mammalian Cell Selection

1. Prepare cells on media of choice (DMEM, EMEM, etc.) and modify cells per standard protocol.

2. Once modified, the cells are incubated for 24-48 hours following transfection before exposure to G418 for cell selection.

3. Expose modified cells to G418 (400 µg/ml) in fresh medium for 10-12 days, or until resistant colonies are visible in culture without the need for microscopy.

Note: Media may be changed every 3 days to ensure availability of nutrients.

For Yeast Cell Selection

1. Prepare YP media consisting of:
    a. 1% bacto-yeast extract (w/v)
    b. 2% bacto-peptone (w/v)
    c. 2% bacto-agar (w/v)

2. Prepare YPD-G418 media by adding the following to YP media:

a. 2% dextrose
b. 200 µg/ml G418

Tips

- The concentration of G418 will have to be optimized depending on the cell type and growth conditions.
- Ammonium sulfate interferes with G418 selection.

Associated Products

- G418 Sulfate (GoldBio Catalog # G-418)

References

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