Protocol

Creation Date: 4/22/2018
Revision Date: 6/14/2019

BaF3 Proliferation Bioassay Protocol

Adapted from D. Ornitz

Introduction

The ^{3}H-thymidine (^{3}H-TdR) incorporation assay is a widely accepted and effective method used to measure DNA synthesis in cells and tissues. In this assay, ^{3}H-thymidine, a radioactive nucleoside, is incorporated into new DNA during the S-phase of the cell division cycle. This assay is routinely used to study the effects of various signaling proteins, including fibroblast growth factors (FGFs), on the induction of mitosis (mitogenesis). FGFs are secreted molecules expressed in almost all tissues and bind to different tyrosine kinase FGF receptors (FGFRs) throughout the cell and nucleus, affecting important processes including embryonic development, tissue repair, maintenance and metabolism. Thus, study of FGF function and receptor binding is a primary research focus and requires reliable mitogenic assay methods. Ornitz et al. effectively used the ^{3}H-thymidine (^{3}H-TdR) incorporation assay in their study of receptor binding specificity of FGFs in engineered BaF3 cells (murine interleukin-3 dependent pro-B cell line) expressing the receptors Fgfr1c or Fgfr2c. Here, we describe the use of ^{3}H-thymidine (^{3}H-TdR) incorporation assay in BaF3 cells.

Materials

P/S+βME Stock Solution:

- 10 ml 100X P/S stock solution
  Penicillin G Potassium = 10,000 U/ml (GoldBio Catalog # P-303)
  Streptomycin Sulfate = 10 mg/ml (GoldBio Catalog # S-150)
- 3.5 µl βME (14.2mM, MW 78.13 g/mol). The final concentration in culture media is 50nM.

Culture Media:

- RPMI 1640 435 ml
- Bovine Calf Serum (BCS) 50 ml (Use **NEWBORN** bovine calf serum, not fetal BCS)
- 100X P/S+βME 5 ml
- 200mM L-Glu 10 ml

+ Add recombinant mouse IL3 to a final concentration of 0.5 ng/ml **Do NOT use Human IL3**
+ For FGFR-expressing BaF3 cells also add G418 (G418 Sulfate, GoldBio Catalog # G-418) 600 µg/ml to BaF3 Culture media.

Assay Media:

- RPMI 1640 440 ml

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- Bovine Calf Serum (BCS) 50 ml (Use **NEWBORN** bovine calf serum, not fetal BCS)
- 200mM L-Glutamine 10 ml

Recombinant mouse Interleukin 3 (GoldBio Catalog # 1310-03):

- Dilute to 500 ng/ml (1000x) in Culture media and freeze aliquots at -80°C.

Method

Baf3 cells were maintained in Culture Media. Briefly, Culture media includes RPMI 1640 media supplemented with 10% newborn bovine serum (BCS), 0.5 ng/ml murine recombinant interleukin 3 (mIL3), 2mM L-glutamine, penicillin/streptomycin (P/S) and 50nM β-mercaptoethanol (BaF3 culture medium), and G418 (600 µg/ml).

1. Wash BaF3 cells and transfected clones twice with Assay media.

2. Resuspend BaF3 cells and transfected clones in Assay media.

3. Plate cells at a density of 30,000 cells/well in a 96 well microtiter plate containing Assay media and 1 µg/ml heparin.

   Note: Total volume in each well should be 150 µl.

4. Prepare 4-fold dilutions of FGFs in Assay media containing 1 µg/ml heparin.

   Note: Dilutions can be made in a 0-5nM range (0, 0.02, 0.08, 0.31, 1.25, and 5nM) and should be made in a volume of 50 µl/well.

5. Add 50 µl diluted FGFs to each well for a total (cell suspension, Assay media, and diluted FGF) volume of 200 µl/well.

6. Incubate cells for 36-48 hours at 37 °C.

7. Add 1 µCi ^{3}H-thymidine in 50 µl Assay media.

8. After 4-6 hours, harvest the cells onto glass fiber filters.

9. Determine incorporated thymidine by liquid scintillation counter (beta plate counter).

10. Refer to your specific beta plate counter manual for cell counting instructions.

Associated Products

- Penicillin G Potassium (GoldBio Catalog # P-304)
- Streptomycin Sulfate (GoldBio Catalog # S-150)
- G418 Sulfate (GoldBio Catalog # G-418)
- Interleukin IL3 (GoldBio Catalog # 1310-03)

References

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