Cell-Based Assay for Hedgehog Signaling with Luciferase Reporter

Reagents

Sonic Hedgehog-conditioned Medium (see below)
Dual Luciferase Assay Kit (Promega)
FBS Culture Medium (FCM; 10% FBS, DMEM, Pen/Strep/Glutamine)
Low FBS Culture Medium (LFCM; 2% FBS, DMEM, Pen/Strep/Glutamine)
CS Culture Medium (CCM; 10% CS, DMEM, Pen/Strep/Glutamine)
Low CS Culture Medium (LCCM; 0.5% CS, DMEM, Pen/Strep/Glutamine)

Procedure

Preparation of Sonic Hedgehog-conditioned medium

1) Culture Shh-N-overexpressing 293 cells to 80% confluency in FCM (5 x 15 cm diameter culture dishes, 25 mL FCM/dish).
2) Remove medium and replace with 25 mL/dish of LFCM. Culture for an additional 24 hours.
3) Collect Shh-conditioned medium by pipetting and transfer to 50 mL centrifuge tubes. Pellet cellular debris by centrifuging at 1,000 rpm (~300 *g)* in a clinical centrifuge.
4) Sterile filter the supernatant using a 0.22-micron filter flasks. This solution is stable at 4 °C for a few months and can also be frozen for long-term storage.

NIH-3T3 cell-based Hedgehog signaling assay

1) Culture NIH-3T3 cells stably containing Gli-dependent firefly luciferase and SV40-*Renilla* luciferase reporters in CCM.
2) When cells are confluent, split 1:5 into 96-well plates (150 μL/well). Culture for an additional 4 days, or until the cells are extremely confluent.
3) Carefully remove medium (the NIH-3T3 cells are prone to detachment at this stage, so the cell monolayer should not be disturbed by aspiration). Replace with LCCM with or without Shh-conditioned medium (typically at a 1:20 dilution; 200 μL/well). Add other pathway modulators if desired.
4) Culture for 30 hours, remove medium, and lyse with 25 μL/well Promega Passive Lysis Buffer. If desired, the cells can be frozen immediately after medium removal or after cell lysis for long-term storage.
5) Transfer 5 μL/well of cell lysate to a luminometer-compatible microplate (e.g. opaque-bottomed plate).
6) Use the Promega Dual Luciferase Kit according to the manufacturer's instructions. Reagents can be used at 25 μL/well to minimize assay costs.