SL pH Titration Microscopy

Chloride and pH calibration in Hek cells

Materials

Ensure the following materials are available before starting the procedure:

- Transfection kit: Qiagen Effectene
- Plasmid DNA: Midi or mini transfection-grade

Procedure

- `Day 1 - Cell Seeding`
  a. Seed 25,000 HEK cells per well in a Labtec chamber with 0.5 mL of culture medium.
- `Day 2: Transfection (6-hour protocol)`
  a. Mix 0.3 µg of DNA with 50 µL EC buffer in a clean microcentrifuge tube.
  b. Add 2.4 µL of Enhancer, vortex gently, and incubate for 5 minutes at room temperature.
  c. Add 4 µL of Effectene, mix thoroughly, and incubate for 10 minutes at room temperature.
  d. Add the transfection mixture to the cells dropwise and incubate for 6 hours.

	1 well	**... wells**
Plasmid DNA	0.3 µg	
Enhancer	2.4 µL	
EC buffer	50 µL	
Effectene	4 µL	

- `Days 3 & 4: Chloride and pH Calibration`
  a. Remove medium from one well carefully.

b. Add 1 mL of the desired buffer containing ionophores (prepared fresh).
c. Incubate the cells for 2 minutes, then change the buffer. Repeat this process 4 times.
d. Begin imaging cells for chloride or pH calibration after buffer changes.
e. For wells that are not being imaged immediately, add a drop of 50 mM Tris (pH 7.3) to maintain the cells.

Notes

- Ensure that buffers with ionophores are freshly prepared and kept at appropriate pH levels.
- Imaging should be performed promptly after the buffer changes to ensure accuracy in pH and chloride calibration.