SL pH Titration Microscopy

Calibration of pH and chloride in Hek cells

Materials

Ensure the following materials are available before starting the procedure:

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

Procedure

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

1. Days 3 & 4: Chloride and pH Calibration
2. Remove medium from one well carefully.
3. Add 1 mL of the desired buffer containing ionophores (prepared fresh).
4. Incubate the cells for 2 minutes, then change the buffer. Repeat this process 4 times.
5. Begin imaging cells for chloride or pH calibration after buffer changes.