SL pH Titration microscopy

pH and chloride calibration in Hek cells :SL:

- Day 1: Cell Seeding

Seed 25,000 HEK cells per well in a Labtec chamber with 0.5 mL of culture medium.

- Day 2: Transfection (6-hour protocol, using Qiagen Effectene)

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

- Mix 0.3 µg of DNA with 50 µL EC buffer in a clean microcentrifuge tube.
- Add 2.4 µL of Enhancer, vortex gently, and incubate for 5 minutes at room temperature.
- Add 4 µL of Effectene, mix thoroughly, and incubate for 10 minutes at room temperature.
- Add the transfection mixture to the cells dropwise and incubate for 6 hours.
- Days 3 & 4: Chloride and pH Calibration
- Remove medium from one well carefully.
- Add 1 mL of the desired buffer containing ionophores (prepared fresh).
- Incubate the cells for 2 minutes, then change the buffer. Repeat this process 4 times.
- Begin imaging cells for chloride or pH calibration after buffer changes.
- 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.