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

- 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)
- 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.
- \cdot 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.

	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
- · 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.