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)

For one well:

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0.3 µg DNA
2.4 µL Enhancer
50 µL EC buffer
4 µL Effectene
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Transfection Steps:

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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.
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- 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:

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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.
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