

Notes on Thawing Spiking HEK Cells:

- Cells not growing well at the initial thaw is typical. Thaw in a 35mm dish and give them enough time to grow up before passaging.
- The time required to reach confluency is highly variable. At most, it has taken up to 4 weeks after the initial thaw before cells could be passaged.
- Replate in high confluency for several passages.
- To help the cells “wake up”, you may wish to remove geneticin and puromycin (used for maintaining the constructs, while penicillin and streptomycin are necessary as antibiotics) and double the serum concentration to 20%.
- After a few passages, cells should begin to proliferate as normal (still slower than typical HEK cells, but that is to be expected with stable integration of two CMV-driven cDNAs).

Growth conditions for Spiking HEK Cells:

- A single monoclonal line was cultured in DMEM/F12 (50:50 mixture), 10% FBS, 1% penicillin (100 U/mL), streptomycin (100µg/ml), geneticin (500µg/mL) and puromycin (2µg/mL).
- Single-cell patch clamp measurements were performed at 10-20% confluence.
- Spontaneously generated action potentials began at ~80% confluence.
- For imaging, cells were grown on a coverglass-bottom dish (P35G-1.5-14-C MatTek), which was pre-treated with Matrigel (BD Biosciences) in a 1:50 dilution in DMEM for 30 min at 37°C.