Cell Plating for Electrophysiology

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Notes: Steps should be done (not necessary if cell stock is only being used for plating cells for electrophysiology) inside of a Biological Safety Cabinet in Sterile conditions.

Procedures (in 35mm dish)**:**

1. Check under microscope to make sure that cells are less than 80% confluent
2. Carefully remove (aspirate) the solution from the T-25 flask containing the cells
3. Rinse with DPBS (Divalent free Dulbecco's Phosphate Buffered Saline) ~ 5 mL
4. Remove (aspirate) the DPBS solution
5. Add 1 mL 0.05% Trypsin EDTA
6. Place in 37° C incubator until cells detach from the dish ~ 5 min
   -Check under the microscope to make sure cells have detached. If the cells are not yet detached, lightly tap the dish against the table.
7. Pipet up and down 5-10 times in the Biological Safety Cabinet
   -Check under the microscope to make sure the cells are dispersed into single cells.
8. Add 2 mL of Cell Media with selection agents into new 35mm dishes
9. Add 1 drop of the trypsinized cells into each 35mm dish with the Cell Media
10. Swirl the dish or pipet up and down
11. If using Kv2.1 T-Rex CHO cells, add tetracycline (1μg/mL) for 1 hour. After 1 hour, replace with fresh media or the external solution for electrophysiology recordings.
12. Label the Dish: Name, Date, Cell Line, Passage #, Ratio

*Cell Media: Ham's – F12 from Gibco, 10% FBS, 1% Penicillin/Streptomycin
To make add 50 mL Media + 50 mL FBS

**Cell Media with Selection Agents (Zeocin and Blasticidin)
F12 from Gibco, 10% FBS, 1% Penicillin/Streptomycin, 1/10,000 Blasticidin, 1/4,000 Zeocin