HEK293 Maintenance

Media:

D-MEM high glucose	435 ml
Fetal Bovine Serum	50 ml
Pen/Strep (100x stock)	5 ml
L-Glutamine (200 mM stock)	5 ml (final concentration 2 mM plus whatever L-Glutamine is provided in DMEM)

Plating:

Remove old media, replace with fresh.

Repeatedly pipet media along growth surface to lift cells. Do not use trypsin, this seems to decrease cell viability.

Dilute cell suspension with fresh media in new flask. A confluent layer spit 1:15 will be ready to split again in one week.

Plate Preparation:

HEK293 cells are only semi-adherent, and can easily lift from the growth surface during assays that require multiple washes (i.e. GABA uptake assays). Coat the surface of plates to be used for such assays with "adhesive" such as poly lysine, laminin, or CellTak. However, of the three, we found laminin to be the most effective.

Poly lysine:

- Obtain PLL or PDL hydrobromide (Sigma P6282 or P6407). Make 0.1 mg/ml solution (working concentration) with sterile water. Store lysine solution frozen at –20.
- Add 0.5-1 ml per well. Incubate at least one hour at 37ºC.
- Aspirate and wash 2x with sterile water.
- Let plates dry in TC hood 30-60 minutes before use. Store extra plates at 4º C.

Transfection:

Plate cells at 5 x 10^5 cells /35 mm dish (2 x 10^5 cells / 12 well) to obtain 90% confluent layer after 24 hours.

Transfect using Lipofectamine 2000 according to the manufacturer's instructions. Briefly, perform transfection in serum-free DMEM, use 4 ug DNA and 10 ul Lipofectamine per 35 mm dish (or 1.6 ug and 4 ul for 12 well). Leave cells in transfection media for four hours, then entirely replace transfection media with fresh standard HEK media. Do not leave cells in transfection media overnight, this results in substantial cell loss.