Fectopro Transfection for Membrane Proteins 2 ml Culture Volume

Reagents:
1.8 mL Expi293 tetR Cells at 3E6 cells/mL
1.5 µg Plasmid (pCDNA 3.1 tet/zeo if inducible)
FectoPro Transfection reagent (Polyplus)
Optimem
HBSS (minus Mg or Ca) with 20 mM Hepes pH 7.4
300 mM valproic acid dissolved in H_2O
40% glucose dissolved in H_2O
0.4 mg/mL doxycycline hyclate in HBSS (only if using an inducible plasmid)

Day 1:
Place 0.2 mL OMEM in an Eppendorf tube
Add 1.5 µg DNA, mix well by pipetting
Add 1.6 µL FectoPro, mix well by pipetting
Incubate at room temperature for 10 minutes
Mix into 1.8 mL of cells at 3E6 cells/mL drop-wise

Day 2: (18-24 hours after transfection)
Make 20 µl (in water) per transfection of:

- 300 mM valproic acid
- 40% glucose

Because filtering wastes a lot of volume, make twice as much as you need, or more.
Filter sterilize into a new Eppendorf using a syringe
Add 5 uM kifunensine if necessary (if the protein is glycosylated)

Day 3: (24 hours after enhancing)
If the protein is in an inducible vector, induce with 20 µl of filter sterilized 0.4 mg/mL doxycycline hyclate in HBSS

Day 4:
Harvest Cells 28-32 hours post induction