hERG Trafficking Assays

hERG is a well known target of drugs that cause cardiac arrhythmia. The typical mechanism of hERG inhibition is through direct blockade of the sodium channel activity. However, compounds have also been discovered that affect hERG activity not by blocking channel activity, but by preventing hERG from reaching the surface in the first place (trafficking inhibitors). Such compounds can also be expected to have different "inhibition kinetics" and not necessarily be detected in conventional hERG activity assays where incubation times may be short. hERG trafficking is also a useful selectivity screen when exploring other trafficking disorders, especially CFTR trafficking in Cystic Fibrosis.

Like CFTR, there are natural mutants of hERG that lead to hereditary long-QT syndromes, and these mutations lead to trafficking defects in hERG, and lower hERG activity. We have developed a hERG trafficking assay similar in format to our CFTR Trafficking Assay for use as a standalone cardiotoxic profiling assay, and also for use on profiling mutant-CFTR corrector compounds. We have created a wild-type and mutant (G601S) hERG with extracellular FAP-tags for easy quantification of surface protein, and Surface/Total protein ratios.

Fluorogen Activating Peptide (FAP) technology combines a genetic tag (to tag the HERG), and a fluorogenic dye that only gives signal when bound to the tag. Using the FAP and cell-excluded and permeant dyes you can selectively label cell-surface and total protein pools in the following wash-free protocol.

### Selective Surface Protein Labeling

A FAP-hERG fusion is created. An N-terminal extension is used to place the FAP in the extracellular space.

Cell excluded fluorogen (↓) labels only the hERG at the cell surface as can be seen from the confocal image at the right.

### Quantifying Total Protein

Cell permeant fluorogen (■) labels both cell-surface hERG and hERG in the secretory pathway.

![Legend:](Legend.png)

Cell excluded fluorogen (emits only when bound to FAP), Unbound extracellular fluorogen (non-fluorescent when free in solution), Cell Permeant Fluorogen (emits when bound), Unbound cell permeant fluorogen (non-fluorescent). Cells are HEK-29


Measurements of FAP tagged hERG at cell surface and total protein level using flow cytometry

Materials
Growth media: DMEM + 10% FBS + 0.1 mM non-essential amino acids (NEAA)

*Bioexpress*
S-1200-500 FBS, US 500 ml

*Fisher Scientific*
SH3024301 DMEM, high glucose, with L-Glutamine/sodium pyruvate 500 ml

*Fisher Scientific*
SH3023801 Non-essential amino acids 100X

*Corning/Cellgro*
25-056-CI Cell stripper, 100 ml

HEK 293 Cells stably expressing FAP-hERG WT or FAP-hERG G601S

Reviving frozen cells
- Thaw quickly in 37°C water bath
- Once completely thawed (5-10 min) add to 5mL of growth media
- Centrifuge at 800 X G for 5min at 4°C
- Aspirate media and resuspend pellet in 5mL of growth media
- Plate in T25 flask or equivalent
- Grow cells in DMEM (high glucose) + 10% FBS + 0.1 mM NEAA at 37C.

Avoid use of antibiotics like pen/strep especially before experiments, however you can use it to grow up enough to archive.
Split culture every 3 days keeping at least 10% of the culture and do not grow past 90% confluency.

Freezing protocol:
- Grow cells until at least 1X10^6 in culture.
- Trypsinize cells and resuspend in growth media.
- Centrifuge at 800 X G for 5min at 4°C.
- Aspirate media and resuspend cell pellet in 1X10^6 cells/1mL of cold freezing media (Growth media + 10% DMSO).
- Incubate cells on ice for 15 min.
- Place in -80°C for at least 24hrs, then transfer to liquid nitrogen for long term storage.

Cytometry Assay protocol:
Day 1: Plate 4X10^4 cells in each well in a 24 well plate. Number of wells: 12. At least one negative control plate should be prepared.

Day 2: Add compounds to cells. Dissolve compounds in 100% DMSO. Dilute compounds in growth media prior to adding to the cell. Each well receives compounds with a final DMSO concentration of 0.1%. For the negative control plate, cells are treated with 0.1% DMSO only.

Day 3: Take a plate out of the incubator 24 hours after compound treatment. Aspirate media off. Rinse cells once with PBS. Add 100 ul cell stripper to each well (Do not use Trypsin which will destroy protein of interest on the cell surface). Mix cells up and down until in single cell suspension state. Split the cell solution into two parts. Add one part (50 ul) to a flow tube containing 450 ul growth media with propidium iodide (PI) and 100 nM se-Red-XC for cell surface protein measurement. Incubate for 2 minutes and read on a flow cytometer. Add the
second part (50 ul) to a flow tube containing 450 ul growth media with propidium iodide and 300 nM se-Red-S to measure the total protein. Incubate for 20 minutes and read on a flow cytometer with 632 nm excitation laser, and 680 nm emission filter (standard cy5 filters are usually appropriate). PI positive cells should be gated out and removed from analysis.

**Analysis:**
Forward and side-scatter gates are drawn to eliminate small objects and cell debris. This cell population is further gated to include only low PI-channels events (cells with integral cell membranes). We typically capture 10,000 total events, mean fluorescence intensities for untreated and corrector treated cells are compared, and this difference is the basis of the assay window. Between 3 and 6 replicates are run for the positive and negative controls, and 6 wells are run for each test sample. The assay window for cell surface protein (the intensity following se-Red-xc treatment) is generally low, but with low enough variation to achieve an assay Z-prime of 0.5 or greater. Fluorescence intensities for total protein signal (the intensity following se-Red-s treatment) are usually substantially larger (since most of the protein is inside the cells, not at the surface). Compound data is normalized to vehicle treated control data.

Using this assay we have recapitulated the data in Van Goor et al., (2011) PNAS 108:18843-18848. We show that VX-809 does not facilitate the maturation of mutant hERG or WT hERG, while VRT325 increases the cell surface pool of mutant hERG and shows a moderate effect on WT hERG maturation.