FluxOR hERG Assay

Background: hERG (human Ether-a-go-go-Related Gene, KCNH2) is a gene encoding alpha-subunit of a potassium channel. Properly formed and functional potassium channels are essential for normal electrical activity in the heart. Interference with the potassium channel due to the drug inhibition can result in acquired long QT syndrome, life-threatening conditions, and even cardiac death. In the past few years, several drugs were withdrawn from the market due to safety issues linked to hERG inhibition. Because the hERG potassium ion channel can accept molecules of many different chemotypes, a broader number of molecules can block its function. This is why a functional screening to identify and remove potential hERG inhibitors is a pivotal step in early stages of drug development.

Service Details: For high-throughput screening of potassium ion channel and transporter activities we use the FluxOR™ II Green Potassium Ion Channel Assay (Thermo Fisher Cat# F20016) and HEK293-hERG cells (human embryonic kidney cell line stably expressing hERG). The test can be carried out in Poly-D-Lysine-coated 96- or 384-well microplates. For the assay, the culture medium is removed and the cells are incubated with the Loading Buffer, followed by incubation in the Assay Buffer and the FluxOR™ II Background Suppressor. The cells are then treated with drug or controls for 30 min. DMSO, used as a negative control, and reference compounds Dofetilide and Haloperidol are run with every experimental batch to verify validity of the test. Then, the Stimulation buffer containing Thallium is added to each well and the intracellular fluorescence is measured at the spectrum for extinction at 460-490 nm and emission at 520-540 nm in a kinetic assay using FLIPR Tetra cellular screening system (Molecular Devices). The assay conditions, the cell density, as well as the concentrations of the reference compounds were optimized in-house for the best performance of the assay.

Deliverable: The results are expressed as % of inhibition, compared to the positive control. Full study report is provided.

Sample Submission: A minimal accurately weighable quantity of dry compound (~1 mg or 2 µmol) or 40 µL of 10-20 mM stock DMSO solution is required for this assay. For multiple assays, lesser amount of compound per assay may be sufficient, depending on the particular project.