Assay Design and Validation

Through the advanced, HTS-compatible instruments, the HTS facility supports a wide variety of detection modalities for assay measurements including: absorbance, fluorescence, time-resolved fluorescence, fluorescence polarization, fluorescence resonance energy transfer (FRET), time-resolved FRET, luminescence, bioluminescence resonance energy transfer (BRET) and scintillation proximity assays (SPA). Cell-based and cell-free assays in experimental systems that have been used in prior models have included but are not limited to; recombinant proteins, membrane fractions, cellular isolates, cell lysates, cell lines, and whole model organisms (e.g., zebrafish, C. elegans, and yeast).

The HTS facility is proficient at helping research investigators develop HTS-compatible assays, obtain preliminary data for grants and funding opportunities, and to screen for the identification and investigation of new compounds for basic research and pharmacological discovery. Discoveries include novel modulators of G-protein coupled receptors, ion channels, transporters, proteases, oxido-reductases, cellular adhesion proteins, lipases, growth factor receptors, proto-oncogenes, protein-protein interactions, DNA-protein interactions, and other molecular targets and pathways that are important in numerous diseases such as neurodegenerative diseases, cardiovascular diseases, malaria, bacterial and viral diseases/illnesses, diabetes, cancer, and HIV.

Typically, assays are validated manually by individual laboratories and evaluated to determine the level of automation and instrumentation needed for HTS services. Once the assay is validated with positive and negative controls and the robustness of the assay (Z-factor/Z') is determined the assay is ready to be automated. During validation of the automation, manual steps are replaced systematically with instruments to ensure accuracy and precision/fidelity of the assay as observed in the manual process. For example, manual pipetting into a 384-well plate can be replaced with use of a Thermo Multidrop, which dispenses liquid into an entire plate evenly and accurately in seconds. Another example is the Velocity11 Bravo liquid handler, this device can aspirate liquid from an entire plate, wash it, and add a solution to a plate very precisely in a matter of minutes. After determining the sequence of events and instrumentation for the assay, a robotic sequence called a schedule is created to integrate these assay procedures into an automated fashion.

Software determines the amount of time each sample will take during individual movements of the robot and the composite time for all samples to be assayed. Testing of the schedule begins with running the schedules for individual instruments, followed by testing of all instruments without reagents, and finally, testing of all instruments with water. Next, an experiment is run using all instruments and robotics integrated by the schedule with experimental controls. After the biological procedure and the instrumentation are validated, the assay is ready for compound screening. The growing library available for screening includes over 160,000 compounds.

Keywords: FRET, BRET, fluorescence, scintillation proximity assay, fluorescence polarization, time-resolved fluorescence, absorbance, pharmacological discovery, compound screening, compound distribution

Assay Targets:
- GPCR Modulators
- Ion Channel Modulators
- Cardiovascular Zebrafish Models
- Diabetes Targets
- Anti-Malarial Targets
- Foodborne Illness Targets
- Coagulation Inhibitors
- Transcriptional Modulators
- COX Inhibitors
• Choline Transporters
• Obesity and Cachexia Regulation Target
• Colon Cancer Targets
• Pancreatic Cancer Targets
• Lung Cancer Targets
• Breast Cancer Targets

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