G-protein coupled receptors (GPCR) are the largest class of cell-surface receptors and are targets for almost 40% of all drugs. Lead discovery, testing, and profiling of efficacious drugs (in the area of cardiovascular diseases and other fields), and understanding of mechanism of action of drug candidates requires assays that can measure the binding of ligands to the receptors, receptor agonist, or antagonist/antagonist, and/or internalization. Accordingly, there is a need for robust and sensitive assays of this type that are suitable for high throughput screening. The Tag-lite cellular screening platform was designed to increase the flexibility of cell-based receptor screening. This platform is ideal for primary and secondary screening and can be applied to a variety of assay formats for pharmacological characterization and screening. Figure 1 illustrates the assay workflow of one of the Tag-lite assay platform with SpectraMax® Paradigm plate reader for characterization of GPCR agonist and antagonists in Tag-lite receptor ligand binding assays, including Dopamine D3, Glucagon GLP-1, and Mu opioid assays. Excellent assay performance was observed as measured by W and assay window values. We also demonstrated performance for cAMP detection, pERK and pAKT kinase assays.

Dopamine D3 Binding Assay: Dopamine receptors are a class of G protein-coupled receptors that are prominent in the vertebrate central nervous system. Dopamine receptors are implicated in various pathological processes, including pleasure, cognition, memory, and fine motor control. Abnormal dopamine receptor signaling is implicated in neuropsychiatric disorders, thus dopamine receptor antagonists represent a valuable tool for the treatment of these diseases. The Tag-lite platform allows one to efficiently label a protein of interest on a variety of assay formats for pharmacological characterization and screening. Figure 2 illustrates the assay workflow of one of the Tag-lite assay platform with SpectraMax® Paradigm plate reader for characterization of GPCR agonist and antagonists in Tag-lite receptor ligand binding assays, including Dopamine D3, Glucagon GLP-1, and Mu opioid assays. Excellent assay performance was observed as measured by W and assay window values. We also demonstrated performance for cAMP detection, pERK and pAKT kinase assays.

Glucacon GLP-1 Binding Assay: GLP-1 receptor is known to be expressed in pancreatic beta cells. Activated GLP1R stimulates the adenyl(c) cyclase pathway which results in increased insulin synthesis and release of insulin. Consequently GLP1R has been suggested as a potential target for the treatment of diabetes. GLP1 is also expressed in the brain where it is involved in the control of appetite and suggested to be involved in mechanisms of memory and learning. The Glucagon GLP-1 receptor ligand binding and competitive inhibition assays were evaluated on the SpectraMax Paradigm system. Excellent assay performance was observed as measured by W and assay window values. We also demonstrated performance for cAMP detection, pERK and pAKT kinase assays.

Results and Discussion

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Optimization of instrumental settings for SpectraMax Paradigm and SpectraMax M5e

Delay time and Integration times were modified during optimization. Greater assay window was achieved when shorter delay and integration times were used for both instruments. Figure 3 shows the characteristic curves for Dopamine D3 and Glucagon GLP-1 assays run on the SpectraMax Paradigm and SpectraMax M5e plate readers. The optimum settings were found to be 20µs delay, 200µs int.

Figure 3. Characteristic curves (left) and plots of the assay window value W for various delay and integration times (right) for the SpectraMax Paradigm. The optimum settings were found to be 20µs delay, 200µs int.

Figure 4. Binding assay: H2K23 cells expressing D3 receptor were labeled with Tb Cryptate, then plated and incubated with appropriate ligands for 1h. Different delay and integration times resulted in greater W and Z’ values.

Figure 5. Competitive inhibition assay: several known dopamine receptor agonists PPHT and bromocriptine or antagonist NAPS. Similar IC50s were observed for the assays run on the SpectraMax Paradigm and SpectraMax M5e plate readers. The assay window and Z’ values for the M5e were 5.3±0.6 and 0.89 respectively.

Figure 2. Results for Mu opioid ligand binding (left) and competitive inhibition assays (right) run on the SpectraMax Paradigm plate reader. The assay window and Z’ values for the M5e were 5.3±0.6 and 0.89 respectively.

Figure 1. Depiction of the Tag-lite cell surface binding assay protocol.

Figure 2. Comparison of W and Z’ values obtained from the Tag-lite paradigm and SpectraMax M5e plate reader platforms.

Figure 3. Comparison of W and Z’ values obtained from the Tag-lite paradigm and SpectraMax M5e plate reader platforms.