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Analysis of GPCR Activity on the EIDAQ™ 100 High Throughput Microscopy System

This application note describes the successful deployment of the NORAK Transfluor™ assay for GPCR activity on the EIDAQ™ 100 High Throughput Microscopy system from Q3DM. The Norak Transfluor™ assay measures GPCR activation by the formation of GFP labeled pits, or vesicles, depending on the GPCR that is being expressed by a cell. The EIDAQ™ 100 is a novel High Throughput Microscopy (HTM) system that delivers accurate, quantitative, imaging, and analysis of cell populations, at high speeds, directly from microtiter plates. For accurate reading/measurement, the EIDAQ™ 100 quantifies distribution of fluorescence emanating from the GPCR associated arrestin-GFP fusion protein and in sub-cellular compartments. Definitive measurements are quantified with a proprietary, multi-scale vesicle definition algorithm developed at Q3DM.

Background and Significance

GPCRs mediate the activity of cell surface receptors and the transduction of a myriad of intra-cellular responses. GPCRs have proven to be a highly amenable class of targets for successful therapeutic intervention. In fact, of the approximately 500 drugs currently on the market today, more than 30% are mediated through the activation of GPCRs. Because GPCRs are membrane-bound proteins, they have been difficult to study in cell extracts, or to isolate and characterize. To directly ascertain GPCR activity, intact cell-based assays are quickly becoming a method of choice in high-throughput screening.

GPCRs transduce extracellular signals through the formation of protein complexes that effect both activation and subsequent desensitization of a cell surface receptor. Agonist binding to a receptor at the cell surface initiates a conformational change in the intracellular domain of the receptor that results in the phosphorylation of the receptor and subsequent binding of arrestin to the receptor. The arrestin-receptor complex is then transported to clathrin-coated pits and internalized to clathrin-coated vesicles. Finally, the entire complex is delivered to the endosomes. Some GPCRs dissociate from arrestin at or near the plasma membrane, while others remain associated and traffic into endocytic vesicles.

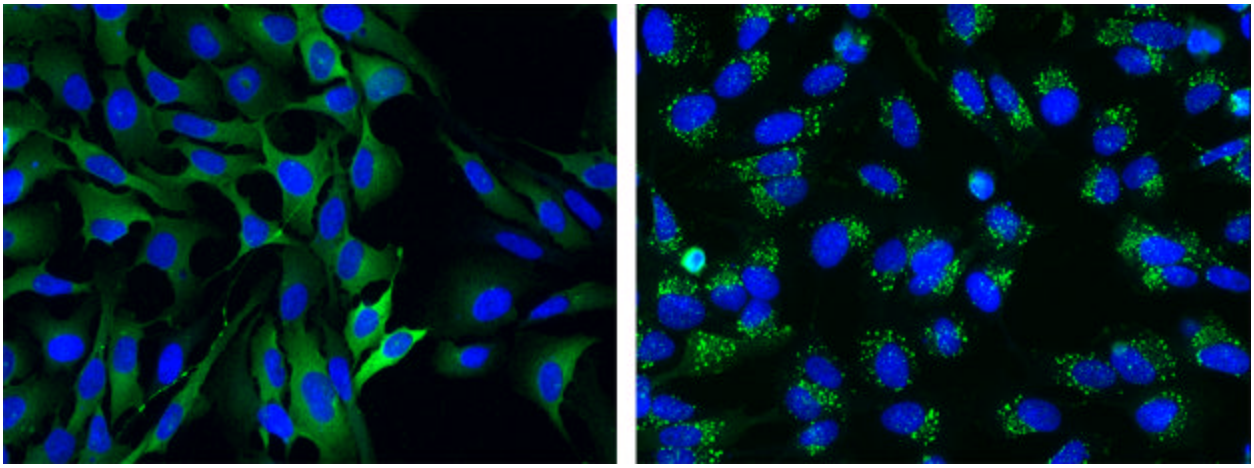

Figure 1. *Vesicle response in human osteosarcoma cells (U2OS). Example 20X 0.5 N.A. fluorescent micrographs of the Norak Transfluor™ assay vesicle response using the EIDAQ™ 100 High Throughput Microscopy (HTM) system. GFP expression (green) and Hoechst staining (blue) are visualized before (left) and after (right) vesicle formation.*

In the Norak Transfluor™ assay, a cell line is developed to monitor the interaction of a given GPCR and a GFP fused to ß-arrestin. When each GPCR is activated, the ß-arrestin will bind to the membrane associated GPCR. The activated ß-arrestin-GPCR complex then enters clathrin-coated pits and migrates to intracellular vesicles via the endosomal pathway. Some GPCRs retain the arrestin molecule throughout this process, so that vesicles will fluoresce with GFP. Other GPCRs will dissociate from the arrestin such that the GFP remains with the pits, or is released to the intracellular space, and the receptors recycle back to the cell surface and bind arrestin again.

For example, in cell lines expressing either the dopamine D1A receptor (D1AR) or the alpha 1b-adrenergic receptor (α1bAR), arrestin-GFP remains localized at the plasma membrane in pits. In contrast, arrestin-GFP is internalized into endocytic vesicles with the vasopressin V2 receptor (V2R) upon ligand binding. The Norak Transfluor™ assay has 2 outcomes, depending on the specific GPCR being assayed. Where gross vesicle response may be analyzed with low resolution optics, meaningful edge/pit response requires sub-micron resolution, which was achieved in this study with the EIDAQ™ 100 HTM system. This detailed image quantification provides valuable insight for use in drug discovery.

Experimental Results and Automation

The combination of automated sub-micron imaging, proprietary image processing and the Norak Transfluor™ assay enables accurate measurement of GPCR activity through vesicle response and pit formation. Validated results of vesicle response and pit formation using the EIDAQ™ 100 HTM system are presented with fluorescent images and dose response curves.

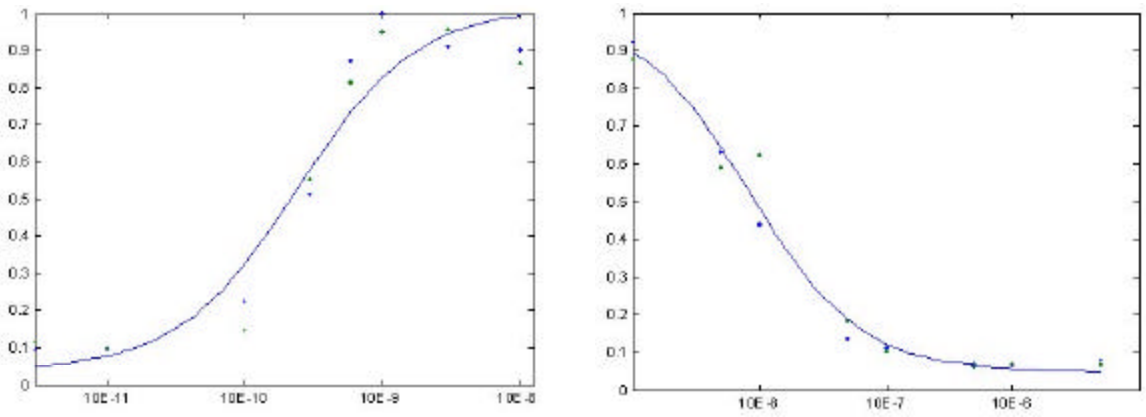

Figure 2. *Dose response of GPCR activation. Agonist induction (left, EC50 = 0.242 nM) and antagonist inhibition (right, IC50 = 8.386 nM) of GPCR response with respect to dose(M). Fluorescent response is normalized and a cell based proprietary metric developed by Q3DM Inc.*

In the example images, GFP expression is visualized in green while the nuclear Hoechst stain is seen in blue. Response measurements were obtained from human osteosarcoma cells (U2OS) grown, dosed and imaged in 96 well-plate format.

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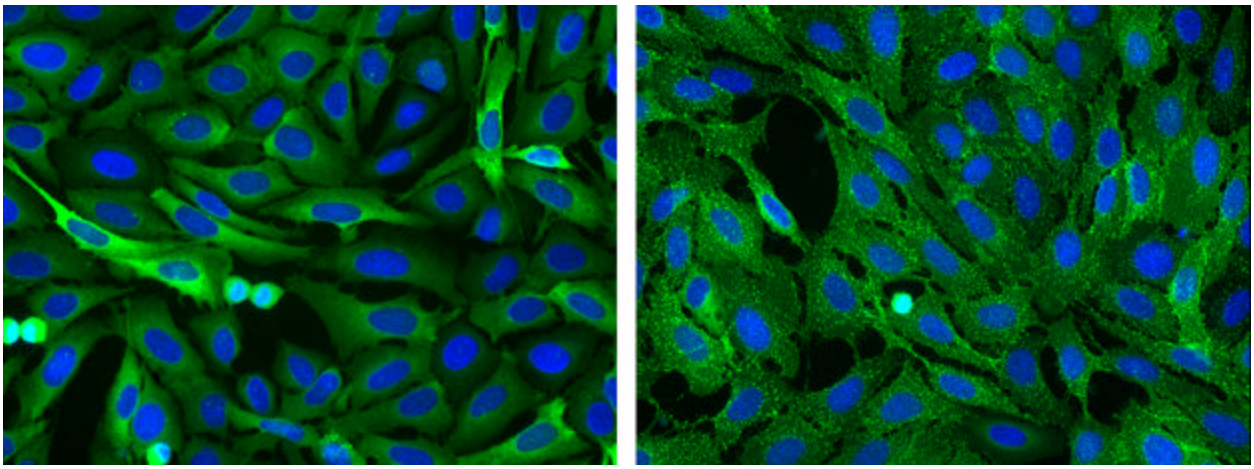

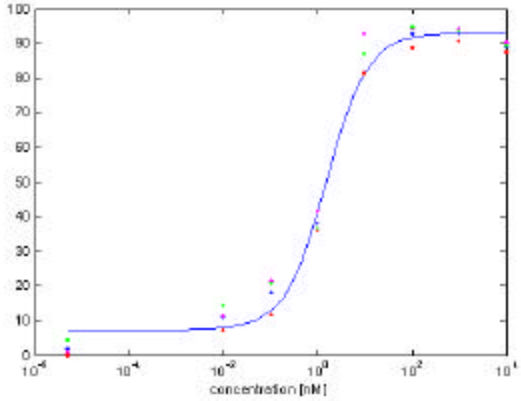

Figure 3. *Pit formation in human osteosarcoma cells (U2OS). Example 20X 0.5 N.A. fluorescent micrographs of the Norak Transfluor™ assay pit formation using the EIDAQ™ 100 High Throughput Microscopy (HTM) system. GFP expression (green) and Hoechst staining (blue) are visualized before (upper left) and after (upper right) pit formation. Dose response curve (right) shows fluorescence response with respect to isoproterenol concentration(M) as derived from 20X 0.5 N.A. images. EC50 = 1.49 +/- 1.01nM.*

Conclusion

Q3DM's proprietary metric FLIV (fractional localized intensity in vesicles) provides a sensitive and accurate measurement of GPCR activation, and enables the rapid and precise quantification of fluorescent signals imaged by the EIDAQ™ 100 High Throughput Microscopy system. Use of the EIDAQ™ 100 in combination with the Norak Transfluor™ assay presents a powerful quantification tool for GPCR activity.

The EIDAQ™ 100 automated High Throughput Microscopy (HTM) system from Q3DM Inc. delivers an unmatched combination of speed, accuracy and detail to quantitative imaging and analysis of cell populations. The EIDAQ™ 100 is used to accelerate drug discovery, for clinical diagnostics, and in basic research.

* Access to the Norak Transfluor™ assay requires a separate patent license, which can be secured from Norak Biosciences, Inc. (Tel. 919-248-8000) for an additional fee. Q3DM Inc. does not have the right or authority to grant any license under patents covering Transfluor™. No license to make, use, sell, offer for sale, or import Transfluor™ is granted by the purchase or license of Q3DM Inc. instrumentation or software. U.S. Patents. 5,891,646, 6,110,693. Other patents pending.

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