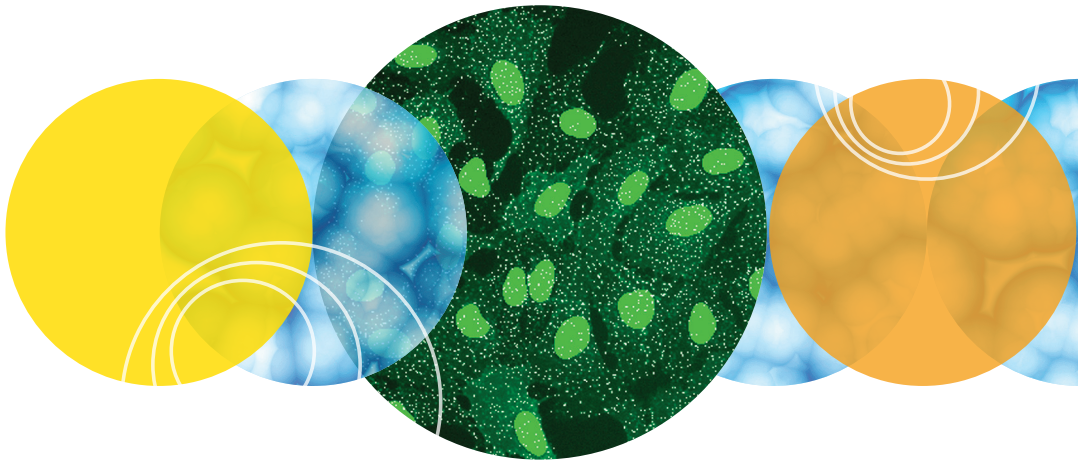


Transfluor Technology

Cell-based fluorescence technology for known and orphan GPCRs

KEY FEATURES

- **Works with all GPCRs—known, orphan, Gi, Gs, Go, Gq**
- **Single readout for all GPCRs eliminates the need for multiple assays**
- **No GPCR labeling or tagging**
- **Requires no prior knowledge of interacting G-protein**
- **Ideal for high-content screening (HCS) analysis**
- **Validated in over 100 GPCRs (from all classes)**

Introduction

The Transfluor® Assay is a cell-based fluorescence assay from Molecular Devices used to screen G-protein-coupled receptors (GPCRs), ligands, and other potential drugs that regulate GPCRs. This patented technology provides a powerful functional assay for detecting a compound's activity against known and/or orphan GPCR targets. The Transfluor Assay is optimized for HCS, secondary screening, and lead optimization.

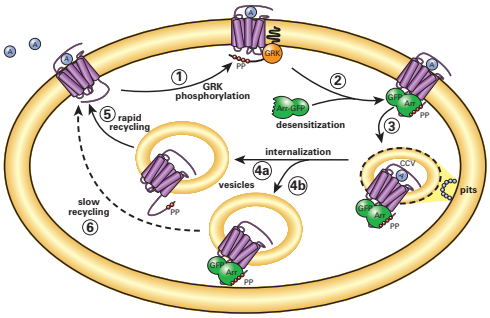
The technology is based on the discovery that, upon activation by ligand binding, virtually all GPCRs rapidly undergo deactivation or "desensitization" by a common pathway. An early step in this pathway is the binding of the cytoplasmic protein, beta-arrestin, to the activated receptor. Beta-arrestin binding deactivates the GPCR signalling and begins the translocation of the receptor into the cell where the ligand is removed and the receptor is recycled back to the cell membrane.

By attaching a fluorescent label to beta-arrestin, the location of the receptor-arrestin complex can be monitored. Since desensitization only occurs with an activated receptor, monitoring beta-arrestin translocation and subsequent receptor recycling provides a method to detect the activation of any GPCR.

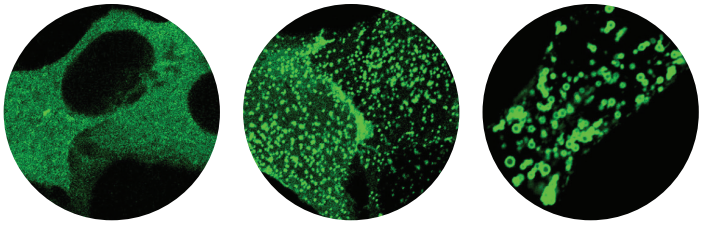
Using cell lines genetically engineered to express both the labeled beta-arrestin and the GPCR of interest, the Transfluor Assay can be used to screen for natural or synthetic ligands, including agonists or antagonists. Activation of the receptor induces a mass movement of the fluorescence to the cell membrane (pits) within seconds, and to endocytic vesicles within minutes.

The Transfluor Assay has been validated as a gold-standard HCS assay by many pharmaceutical and biotechnology companies, and is considered an integral part of their drug discovery process. Transfluor has been successfully validated on over 100 GPCRs and works across all GPCR classes (Class I, II, III), regardless of interacting G-protein (Gs, Gi/o, and Gq).

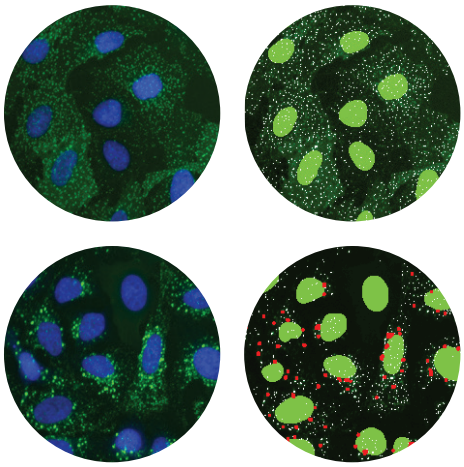
MOLECULAR DEVICES



ß-arrestin-GFP translocation. The Transfluor Assay utilizes the redistribution of beta-arrestin-GFP to monitor GPCR activation and inactivation.



A robust, highly sensitive assay. Left: untreated. Activation of the receptor induces, within seconds, a mass movement of the fluorescence to the cell membrane **(pits, middle)**, and within minutes to endocytic vesicles **(right)**.



Validated on numerous systems. The GPCR Cycling Application Module in MetaXpress Software identifies nuclei (green), pits **(top, white)** and vesicles **(bottom, red)**. Images were acquired with the ImageXpress® Micro System from Molecular Devices.

Orphan GPCRs

The Transfluor Assay requires no prior knowledge of the interacting G-protein. This important feature of the Transfluor Assay makes it ideally suited for screening orphan GPCRs (oGPCR). The Ligand Independent Translocation (LITe) assay is an agonist-independent assay used to verify the translocation of beta-arrestin-GFP in orphan GPCRs.

Transfluor Evaluation Kit

The Transfluor Assay Evaluation Kit enables use of the technology for a limited, one-time, six month evaluation period. The kit provides all the necessary tools for evaluating the technology with the user's own instrumentation and GPCR. This allows users to become comfortable with the technology before proceeding to a tiered licensing option that meets the user's specific needs.

Transfluor Evaluation Kit components

- U2OS ß-arrestin2-GFP cells (Transfluor Assay parental cell line to track translocation and evaluate agonist/antagonist GPCR signaling)
- Transfluor Assay Demo Plate (Imaging grade plate with fixed cells to assist with instrument validation, and image acquisition and analysis)
- Limited Label License
- Complete product documentation

Please contact your local sales representative for detailed information regarding the Transfluor Assay Evaluation Kit.

Tiered licensing options

Use of the Transfluor Assay requires the purchase of a Transfluor Assay Technology Use License from Molecular Devices which is renewable on an annual basis. A scalable license structure is available from research up to high-throughput screening including the following examples:

- Transfluor Assay Research Site License
- Transfluor Assay Research Global License
- Transfluor Assay Screening Site License
- Transfluor Assay Research and Screening Site License

Two additional third-party licenses are required to run the Transfluor Assay: a GFP license (Molecular Devices recommends the Prolume Renilla reniformis (Rr) GFP license) and BioImage's fluorescence redistribution license. Terms are as follows:

- Prolume license is renewable on an annual basis for a nominal fee
- Biomage license is a one-time fee
- Prolume and Bioimage licenses both provide worldwide coverage

To streamline the process, these licenses are available directly from Molecular Devices. Alternatively, they can be obtained directly from Prolume and Bioimage.

Ordering information	Part number
Transfluor Assay Evaluation Kit (Commercial Use)	R8178
Transfluor Assay Evaluation Kit (Non-Commercial)	R8177
U2OS (G418) Rat ß-Arrestin 2-RrGFP Cell Line	TF-U2OS-rBA2GFP

Please contact your local sales representative for detailed information regarding these licenses. In conjunction with the Transfluor Assay license, Molecular Devices also offers a stably transfected GFP beta-arrestin starter cell line and relevant plasmids.

Purchase of these Transfluor Assay products requires a prior execution of the Transfluor Assay License Agreement and the BioImage license. If ordering the GFP cell line or plasmid, a Prolume license is also required for purchase.

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