

## TransfluoR<sup>®</sup> Technology

### Universal

GPCR Assay for Known & Orphan Receptors & All Ligand Types

### Highly Reproducible

### Very Sensitive

### Simple & Low Cost

### High IQ™

Image Quantification Assay

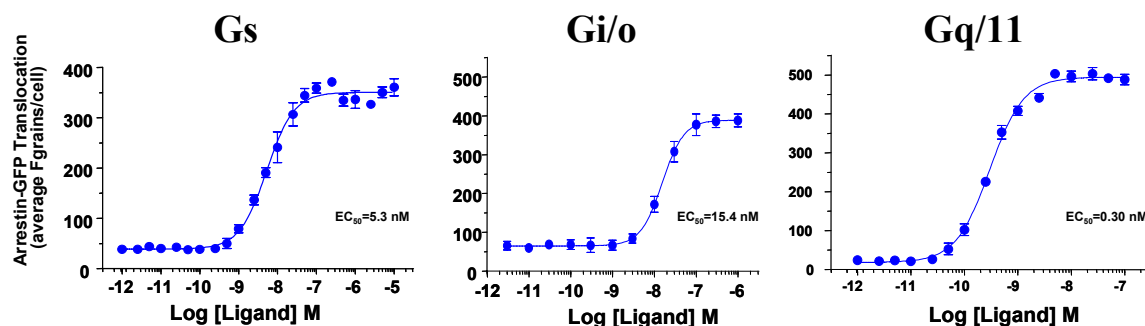
### High Throughput

### High Content

## TransfluoR<sup>®</sup> Technology: A Universal GPCR Assay

TransfluoR<sup>®</sup> is an advanced, cell-based screening technology applicable to all *known* and *orphan* GPCRs. TransfluoR<sup>®</sup> has been successfully validated on over 85 GPCRs, and works across all GPCR classes (Class I, II, III), regardless of interacting G-protein (Gs, Gi/o and Gq/11). TransfluoR<sup>®</sup> eliminates the need for multiple GPCR assay platforms.

The following dose-response curves, representing receptors coupled to different G proteins, illustrate the broad utility of the TransfluoR<sup>®</sup> technology.

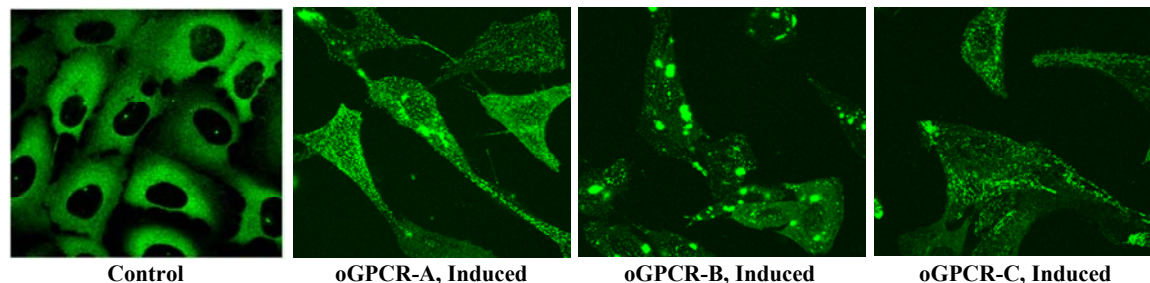


The TransfluoR technology monitors receptor activity by detecting movement of  $\beta$  arrestin-GFP in the cell. A partial listing of GPCRs that have been shown to translocate  $\beta$  arrestin-GFP is included on the back of this sheet.

## Orphan GPCRs

In contrast to current methods of screening GPCRs, the TransfluoR<sup>®</sup> technology is based on the mechanism for termination of GPCR signaling, known as *receptor desensitization*. This mechanism is shared by virtually all GPCRs and is activated by ligand binding. TransfluoR<sup>®</sup> technology requires no prior knowledge of the interacting G-protein. This important feature of the TransfluoR<sup>®</sup> technology makes it ideally suited for screening orphan GPCRs (oGPCR).

Norak has developed a propriety technique to assist in validating orphan GPCR screens. The technology, called LITE™, is an agonist-independent assay used to verify the translocation of  $\beta$  arrestin-GFP in orphan GPCRs. This agonist-independent assay is illustrated below in the pictographs of three orphan GPCRs.





## For Information on Licensing Transfluor®:

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*Transfluor® is protected under United States patents 5,891,646, 6,110,693, 6,770,449 and other pending United States and international patents. A license from Norak Biosciences is required to use the proprietary Transfluor® technology. Transfluor technology includes an arrestin fused to any optically*

## Partial List of GPCRs that Translocate $\beta$ Arrestin-GFP (To Date)

$G_s$	$G_{i/o}$	$G_{q/11}$
<ul style="list-style-type: none"> <li>A2a adenosine</li> <li>A2b adenosine</li> <li><math>\beta</math>1-adrenergic</li> <li><math>\beta</math>2-adrenergic</li> <li>CRF1 corticotropin releasing factor</li> <li>D1 dopamine</li> <li>D5 dopamine</li> <li>FSH follicle-stimulating hormone</li> <li>Glucagon</li> <li>LH luteinizing hormone</li> <li>PTH1 parathyroid hormone</li> <li>E2 prostaglandin</li> <li>E4 prostaglandin</li> <li>Secretin</li> <li>VIP1 vasoactive intestinal peptide</li> <li>V2 vasopressin</li> </ul>	<ul style="list-style-type: none"> <li><math>\alpha</math>2a-adrenergic</li> <li><math>\alpha</math>2b-adrenergic</li> <li><math>\alpha</math>2c-adrenergic</li> <li>A1 adenosine</li> <li>A3 adenosine</li> <li>Apelin</li> <li>C5a anaphylatoxin</li> <li>CCR5 chemokine</li> <li>CXCR1 chemokine</li> <li>CXCR2 chemokine</li> <li>CXCR4 chemokine</li> <li>D2 dopamine</li> <li>D3 dopamine</li> <li>D4 dopamine</li> <li>Edg1 endothelial diff. gene</li> <li>Edg2 endothelial diff. gene</li> <li>Edg3 endothelial diff. gene</li> <li>Edg5 endothelial diff. gene</li> <li>5HT1A hydroxytryptamine</li> <li><math>\delta</math>-opioid</li> <li><math>\mu</math>-opioid</li> <li>MCH1 melanin conc. hormone</li> <li>M2ACh muscarinic acetylcholine</li> <li>E3 prostaglandin</li> <li>N-formyl peptide</li> <li>Neuropeptide FF</li> <li>Somatostatin</li> </ul>	<ul style="list-style-type: none"> <li><math>\alpha</math>1b-adrenergic</li> <li>AT1A angiotensin II</li> <li>CCK-A cholecystokinin</li> <li>CCK-B cholecystokinin</li> <li>Cytomegalovirus US28</li> <li>ETA endothelin</li> <li>GnRH (type2) gonadotropin releasing hormone</li> <li>5HT2A hydroxytryptamine</li> <li>5HT2C hydroxytryptamine</li> <li>m1ACh muscarinic acetylcholine</li> <li>mGluR1 metabotropic glutamate</li> <li>NK1 neurokinin</li> <li>NK3 neurokinin</li> <li>NT1 neurotensin</li> <li>Orexin-1</li> <li>Oxytocin</li> <li>PAR2 proteinase-activated</li> <li>Platelet-activating factor</li> <li>TRHR-1 thyrotropin releasing hormone</li> <li>TRHR-2 thyrotropin releasing hormone</li> </ul>

### Also

- 12 *Drosophila* GPCRs
- Fz4 frizzled receptor
- T $\beta$ RIII transforming growth factor- $\beta$

In addition, there are approximately 25 other known GPCRs that have been shown to translocate arrestin-GFP as part of proprietary research programs (internal and external) at Norak Biosciences.

## List of Host Cells Used for Transfluor® Assay (To Date)

### Stable Transfection

- HEK 293
- U2 OS

### Transient Transfection

- HEK 293
- U2 OS
- K-562
- HeLa S3
- COS-7
- CHO K1