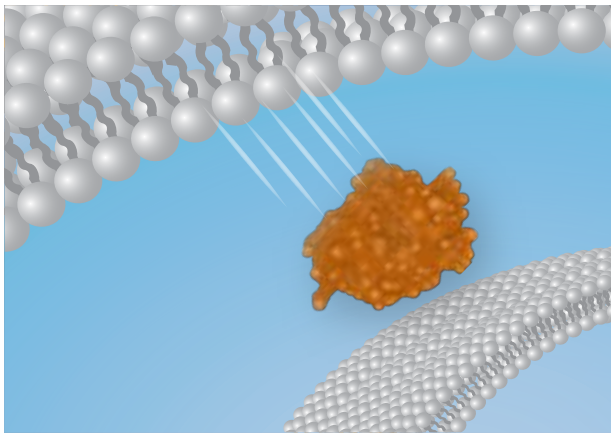
PathHunter®
Protein Translocation Assays

Track Cellular Movement of Proteins to Multiple Membrane Compartments

The movement of proteins between cellular compartments is essential for their specific biological role and the function of the cell. If these protein movements are altered by a ligand, protein mutations, or aberrant signaling, undesirable effects may occur, often resulting in drug tolerance, unwanted side effects, and disease. PathHunter protein translocation cell-based assays provide a high throughput, quantitative non-imaging approach to study trafficking of your protein of interest between different cellular compartments, and analyze the pharmacological effects of compounds on these translocations.



Protein Translocation Assays Highlights

- Explore protein movement using a non-imaging, high throughput cell-based assay platform
- Study pharmacology of ligands that trigger protein translocation to the plasma membrane, endosome, or nucleus
- Create your own quantitative cell-based assays to study translocation of any protein

Cell-Based Assays Products and Services

Products	Stable and rigorously validated cell lines for GPCRs, nuclear hormone receptors (NHRs), receptor tyrosine kinases (RTKs), and ion channels
	Ready-to-use kits for GPCRs and NHRs with cells, plates, and reagents
	Parental cell lines*, vectors, and reagents to make your own cell-based protein translocation assays
Services	High throughput screening and profiling services for GPCRs and nuclear proteins
	Custom assay development services to generate vectors and build clonal cell lines or stable pools in a variety of cell types

* DiscoverX engineered cells (e.g. CHO-K1, HEK 293, U2OS) expressing a reporter protein (plasma membrane, nuclear, or endosomal specific) attached to an enzyme acceptor (EA) fragment used for creating PathHunter enzyme fragment complementation (EFC) assays.

To learn more about our protein translocation solutions, visit discoverx.com/translocation

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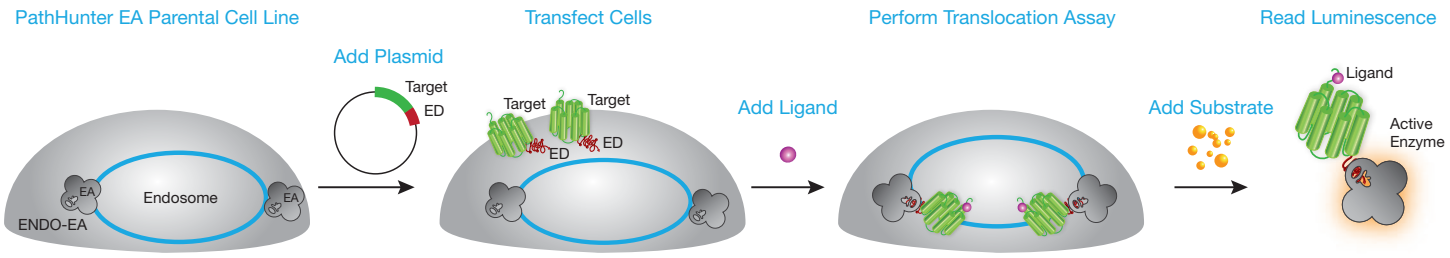
Complete Solutions for Protein Translocation Analysis

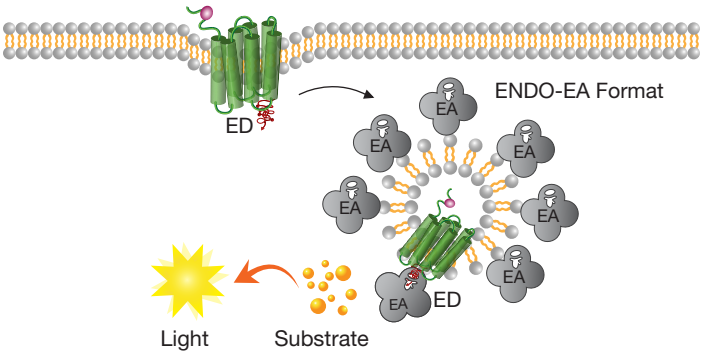
Make Your Own Cell-Based Protein Translocation Assays



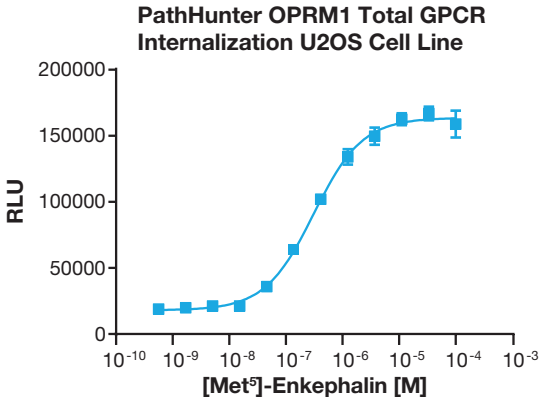
Generate a PathHunter® translocation assay by first creating a plasmid vector with your target protein of choice tagged with an enzyme donor [ED; ProLink™ (PK) or enhanced ProLabel® (ePL)]. Simply transfect this plasmid into a PathHunter EA parental cell line containing an EA-reporter protein (e.g. Endosome-EA [ENDO-EA] as shown in this example), and then perform a translocation assay in the presence of a ligand. GPCR total internalization from the cell membrane to the endosome is shown.

Analyze Membrane Protein Internalization to the Endosome

Identifying ligands that give less receptor internalization is desirable for understanding drug tolerance, unwanted side effects, and tachyphylaxis. Measure internalization of membrane proteins like GPCRs or RTKs from the plasma membrane to the endosome upon ligand stimulation.



Study GPCR membrane protein recycling. Use an ED-tagged GPCR along with an endosome-EA (ENDO-EA) parental cell line to monitor internalization of GPCRs from the membrane to the endosome. Alternatively, use a PathHunter Activated GPCR Internalization Assay (with an ED-tagged endosome and EA-tagged β-arrestin) to study GPCR internalization due to ligand activated β-arrestin recruitment and internalization (not shown here).



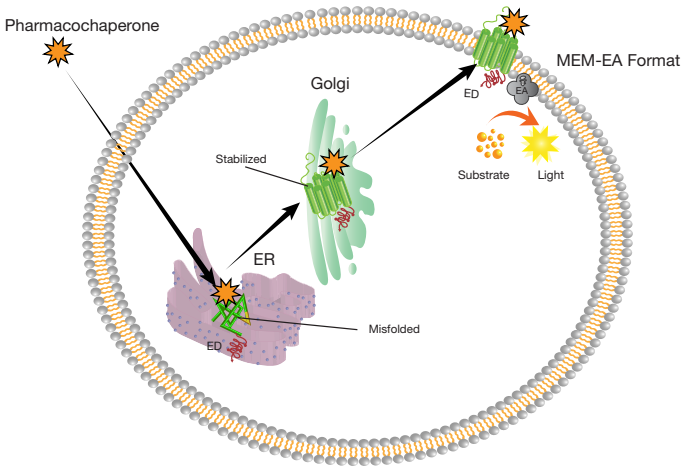
A GPCR internalization assay was made using ENDO-EA U2OS parental cells expressing PK1-tagged opioid receptor mu, OPRM1 (Cat. No. 93-0745C3). The data indicate translocation of the ED-tagged receptor from the plasma membrane to the EA-tagged endosome upon stimulation with agonist [Met^5]- enkephalin (EC_{50} = 589 nM; signal/background (S/B) = 4.3).

To learn more about GPCR internalization assays, visit discoverx.com/gpcr-internalization

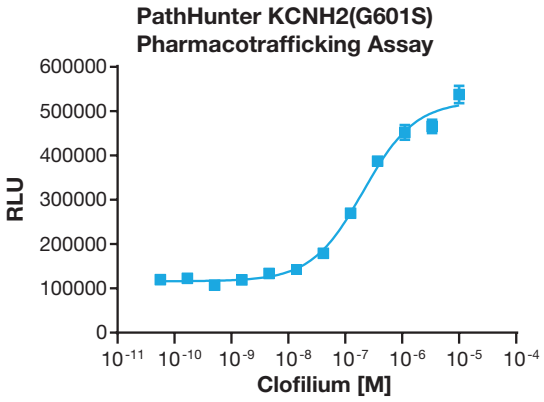
Applications for Cellular Protein Translocation Assays

Discover Pharmacochaperones That Rescue Disease-Associated Mutant Receptors

Identify pharmacochaperone compounds which function by promoting proper folding of misfolded GPCRs or ion channels and assist in trafficking from the endoplasmic reticulum (ER) to the plasma membrane.



Mutations in GPCRs or other membrane proteins can lead to misfolding of the membrane protein and improper trafficking to the membrane. Use an ED-tagged membrane protein along with a plasma membrane EA (MEM-EA) parental cell line to screen small molecule pharmacochaperones that bind and rescue mutant membrane proteins trapped in the ER.



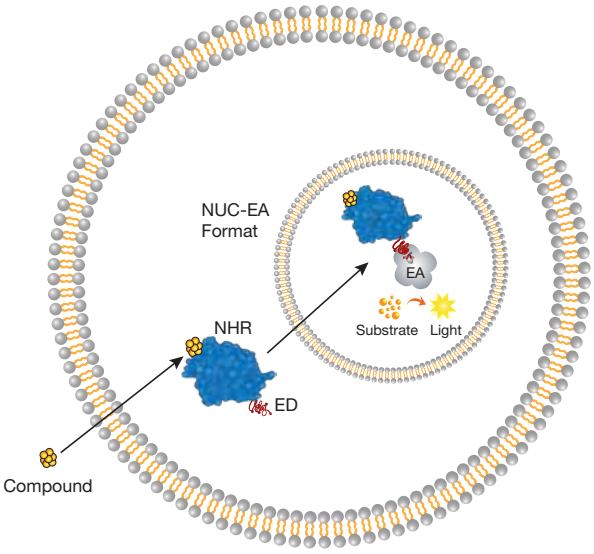
Mutations in the potassium voltage-gated channel human ERG (KCNH2), can lead to reduced functional potassium current, long QT syndrome, and cardiac arrhythmias. A pharmacotrafficking assay was created using MEM-EA U2OS parental cells stably transfected with PK-tagged mutant KCNH2(G601S) (Cat. No. 93-1064C3). The pharmacochaperone clofilium was able to rescue the mutant ion channel by promoting proper folding resulting in successful trafficking from the ER to the membrane (EC_{50} = 564 nM; S/B = 3.5).

To learn more about membrane protein trafficking assays, visit discoverx.com/pharmacotrafficking

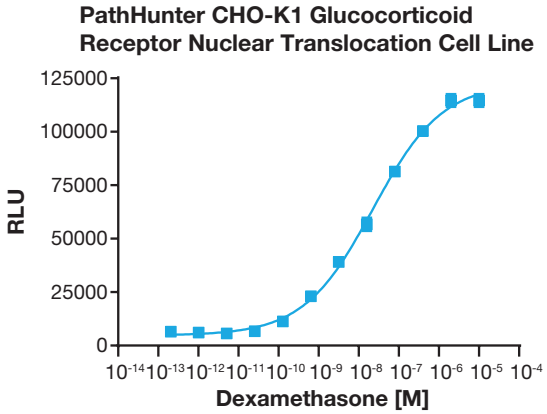
Applications for Cellular Protein Translocation Assays continued...

Quantify Nuclear Protein Translocation to the Nucleus

Obtain quantitative data showing binding and ligand induced translocation of nuclear hormone receptors (NHRs) such as androgen, glucocorticoid, liver X, mineralocorticoid, and progesterone receptors.



Use an ED-tagged NHR along with a nuclear-EA (NUC-EA) parental cell line to analyze compound binding and translocation of NHRs from the cytoplasm to the nucleus.



A nuclear translocation assay was generated using NUC-EA CHO-K1 parental cells stably transfected with PL-tagged glucocorticoid receptor (Cat. No. 93-0002C2). The data indicate successful translocation from the cytoplasm to the nucleus, where EA is localized, upon stimulation of ligand dexamethasone (EC_{50} = 8.2 nM; S/B = 15.9).

To learn more about our protein translocation solutions, visit discoverx.com/translocation

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