



BioInvenu
illuminating drug targets

LinkLight™ Assay Technology:

A Versatile Cell-based Protein-protein Interaction Assay Platform

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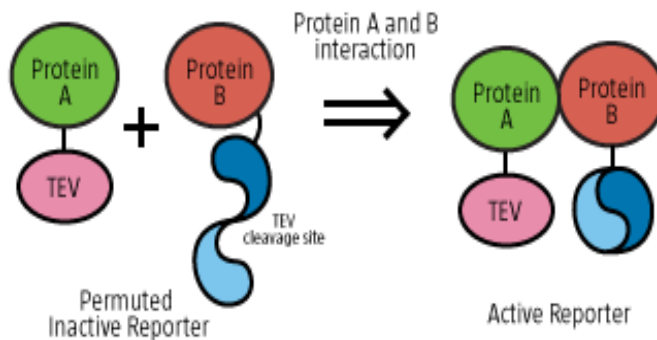
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LinkLight™ Assay Technology

Utilizing protein-protein interactions as functional signal readout

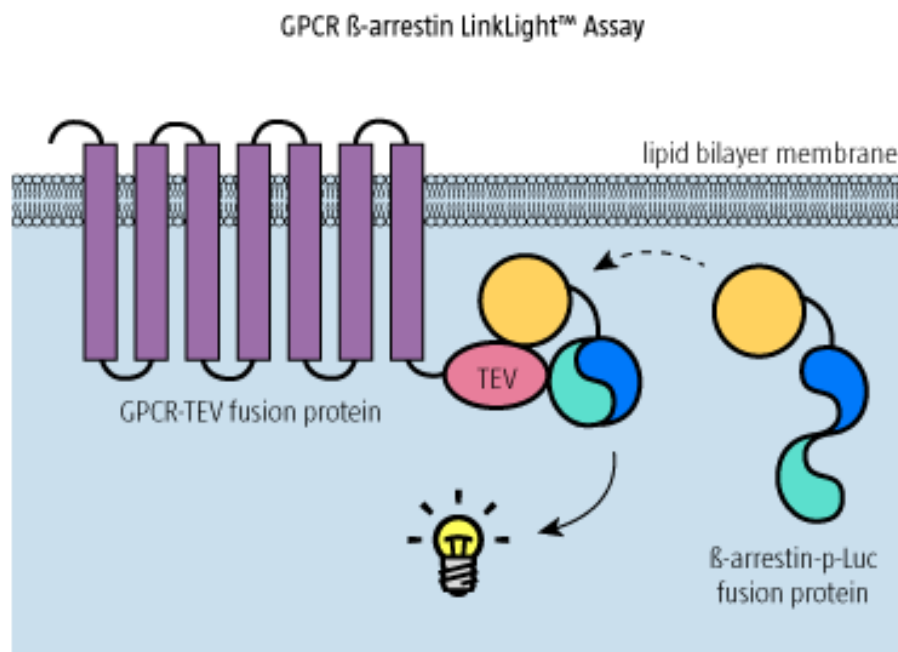


- ❖ Developed in 2006 in Sanofi-Aventis
- ❖ Two provisional patent applications were filed in 2007 & 2008)
- ❖ Patent issued in 2014

LinkLight™ technology consists of two components: a protein A is fused to a Tobacco Etch Virus (TEV) protease and a protein B is fused to an inactive permuted protein containing a TEV protease cleavage site. Upon interaction between protein A and B, inactive permuted protein is cleaved, the cleaved fragments spontaneously refold, and active reporter protein is reconstituted.

GPCR LinkLight™ Assay

Utilizing GPCR & β -arrestin interactions as functional signal readout



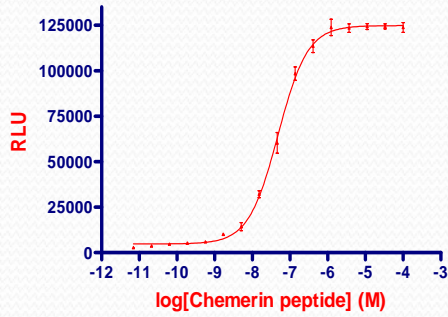
Simple Assay Procedure

1. Seed cells in 384-well plate, culture for 24 hours.
2. Change to serum-free medium if necessary, add ligands, incubate for one hour.
3. Add luciferase detection reagent, count signals on a luminescent reader.

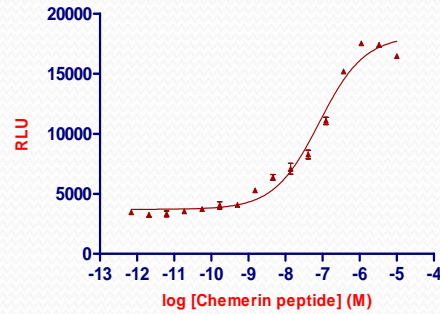
A GPCR is linked with a TEV protease. β -arrestin 2 is linked with a permuted luciferase (pLuc) containing a TEV cleavage site. Upon GPCR recruits β -arrestin 2, the permuted luciferase is cleaved. The cleaved fragment spontaneously refolds to form active luciferase. Highly sensitive luminescent signals produced with the active luciferase are easily detected.

Examples of GPCR LinkLight™ Assays

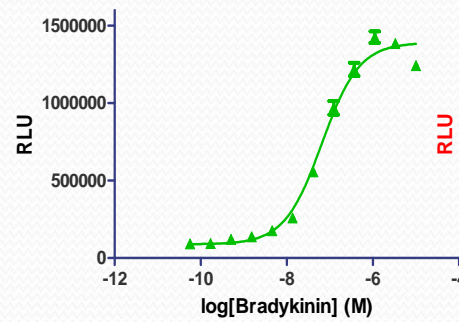
CMKLR1 LinkLight™ Stable cells



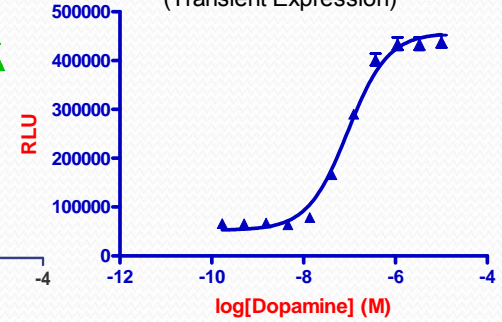
GPR1 LinkLight™ Assay
Monoclonal Stable cells



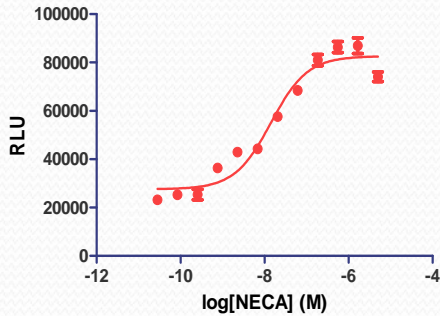
CCKAR LinkLight™ Assay



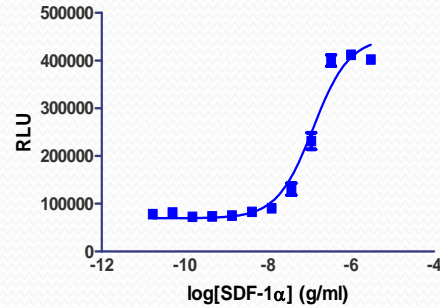
DRD2 LinkLight™ Assay
(Transient Expression)



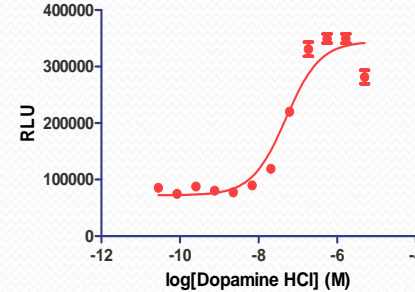
ADORA2B LinkLight™ Assay
(Transient Expression)



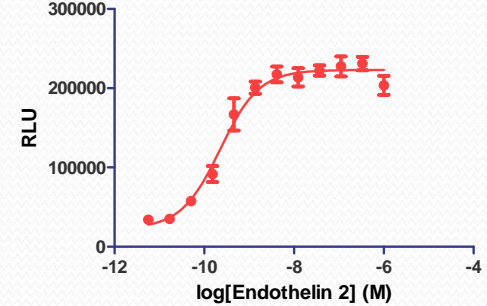
CXCR7 LinkLight™ Assay
(Transient Expression)



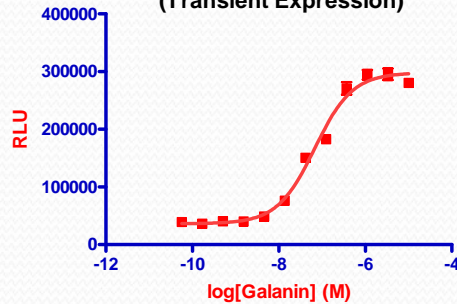
DRD1 LinkLight™ Assay
(Transient Expression)



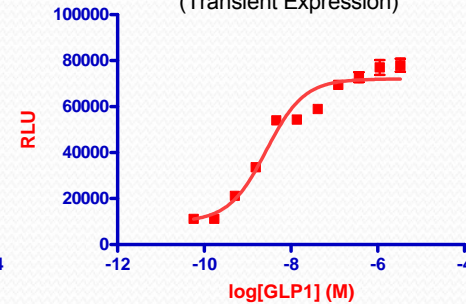
ENDRB LinkLight™ Assay
(Transient Expression)



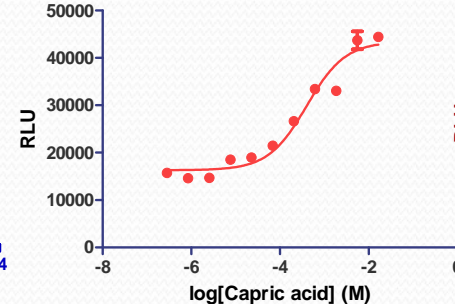
GALR1 LinkLight™ Assay
(Transient Expression)



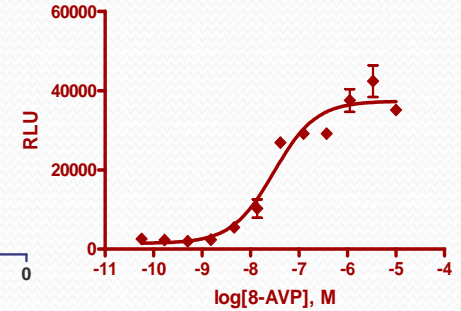
GLP1R LinkLight™ Assay
(Transient Expression)



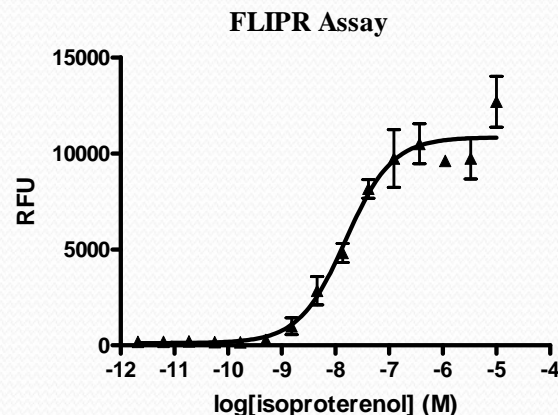
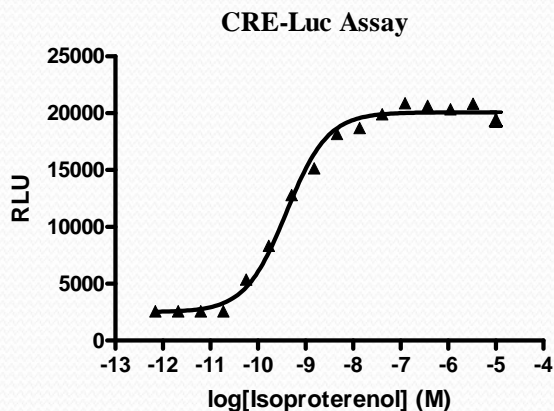
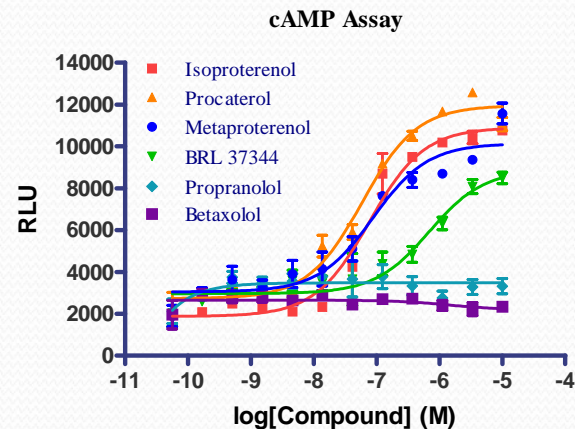
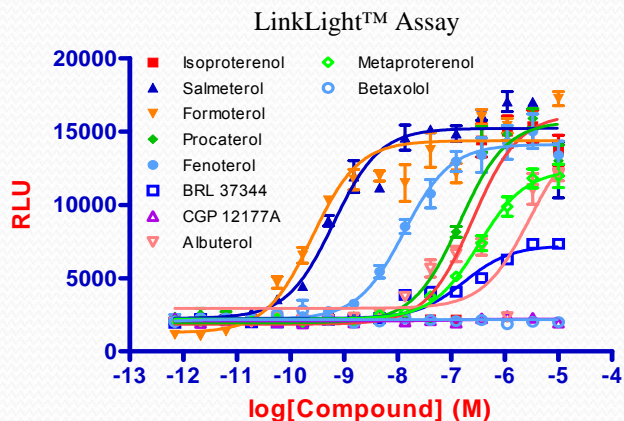
GPR84 LinkLight™ Assay
(Transient Expression)



AVPR2 β-arrestin LinkLight™ Assay



TEV linked GPCR ADRB2 does not alter G-protein signaling pathway

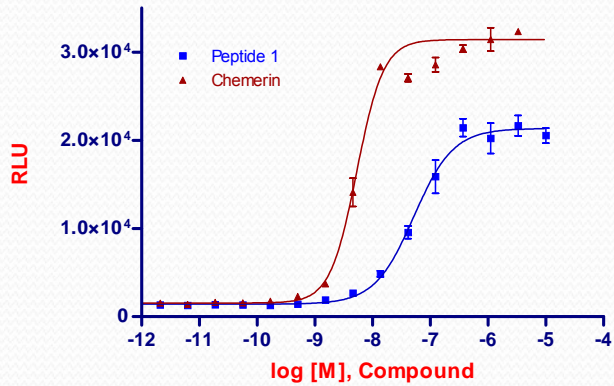


Assay	LinkLight™	cAMP	FLIPR	Cre-Luc
Isoproterenol EC ₅₀	3.5 nM	9.1 nM	7.2 nM	2.0 nM

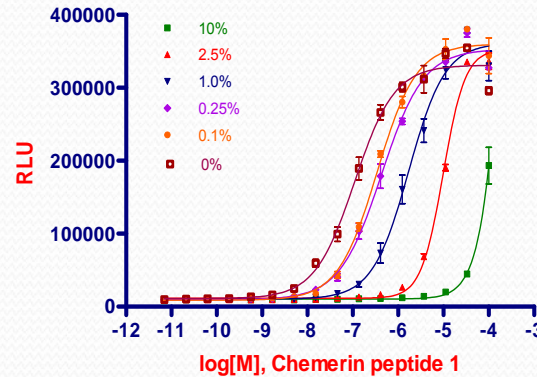
Other TEV linked GPCRs such as GPR43, EDG1, GPR1 have also been demonstrated no interference to G-protein signaling

CMKLR1 LinkLight™ Assay Parameters

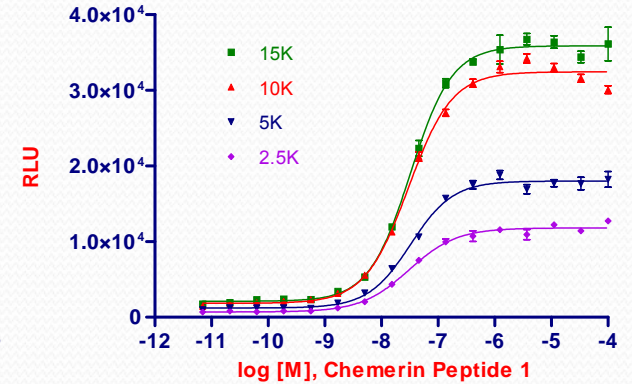
Chemerin vs. Peptide 1



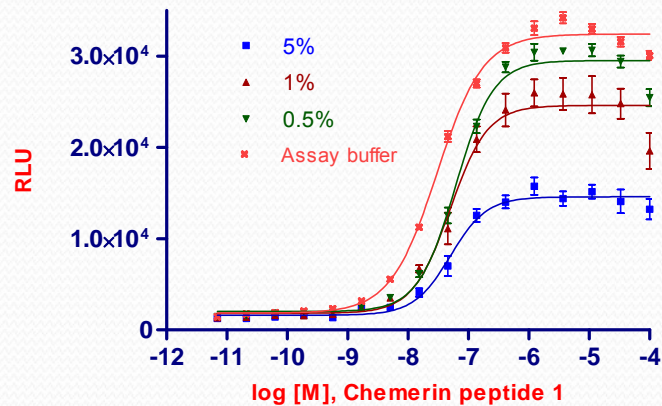
Serum effect



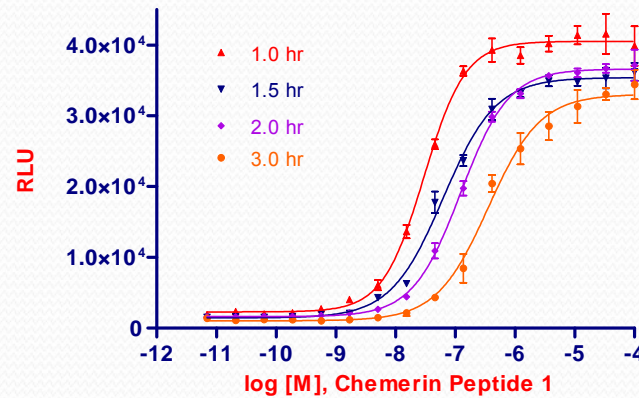
Cell Number Per well



DMSO Concentration



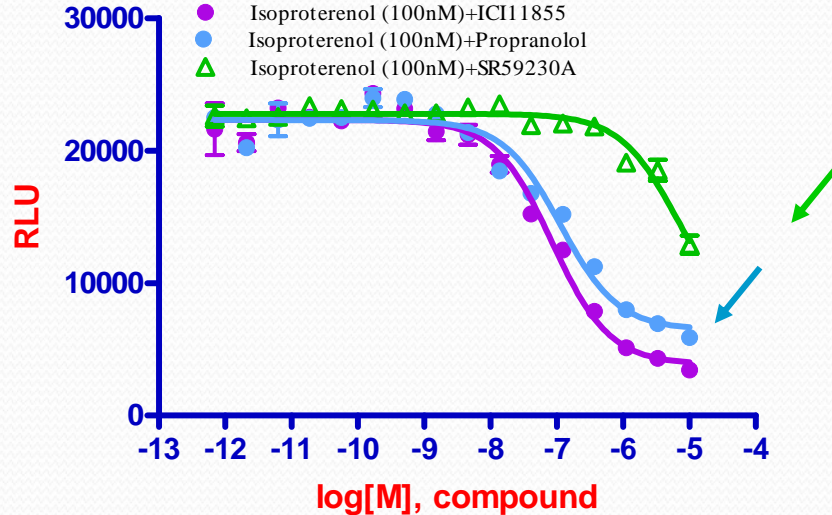
Incubation Time



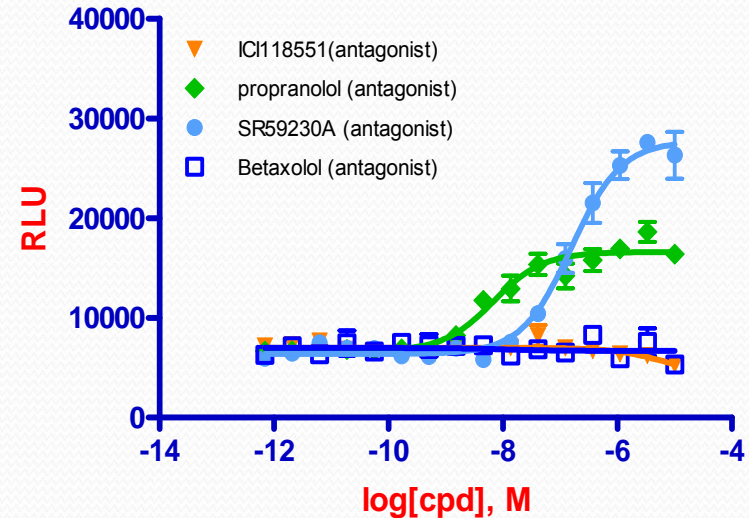
GPCR LinkLight™ Assay

Identify biased ligands

ADRB2 LinkLight™ antagonist assay



ADRB2 LinkLight™ agonist assay



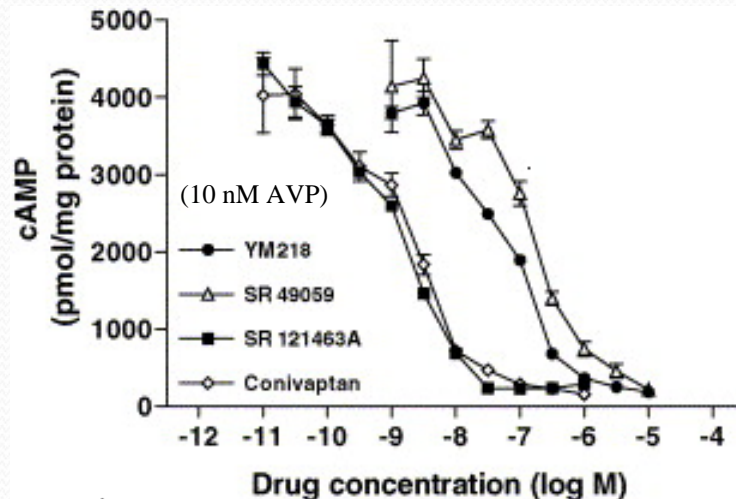
Some of well-known ADRB2 antagonists showed partial agonist activity in GPCR LinkLight™ assays

GPCR LinkLight™ Assay

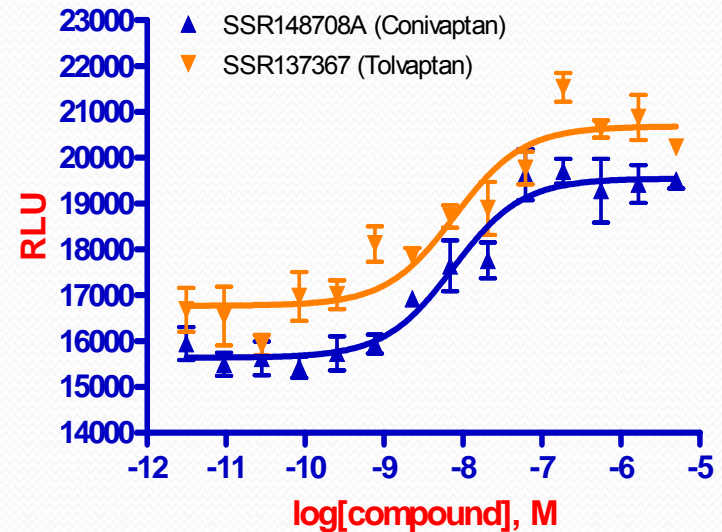
Identify biased ligands

G protein Signaling cAMP assay

Pharmacological Research 51:275-218 (2005)



b-arrestin Signaling LinkLight™ assay



Some of vasopressin 2 antagonists showed weak agonist activity in LinkLight™ assays

Comparison of Cell Based Protein-Protein Interaction Assay Technologies

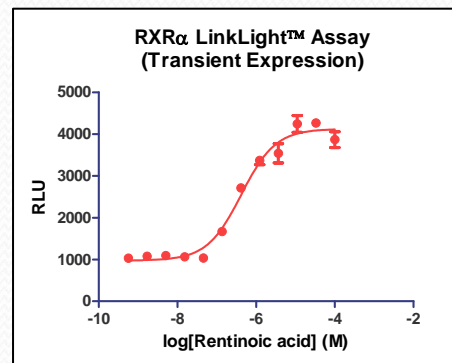
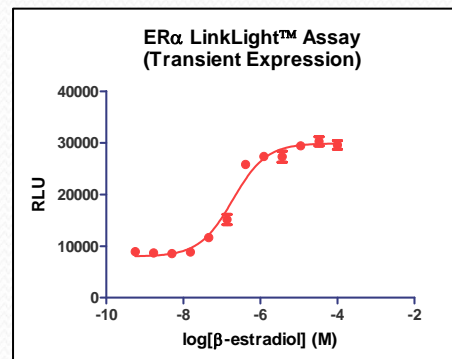
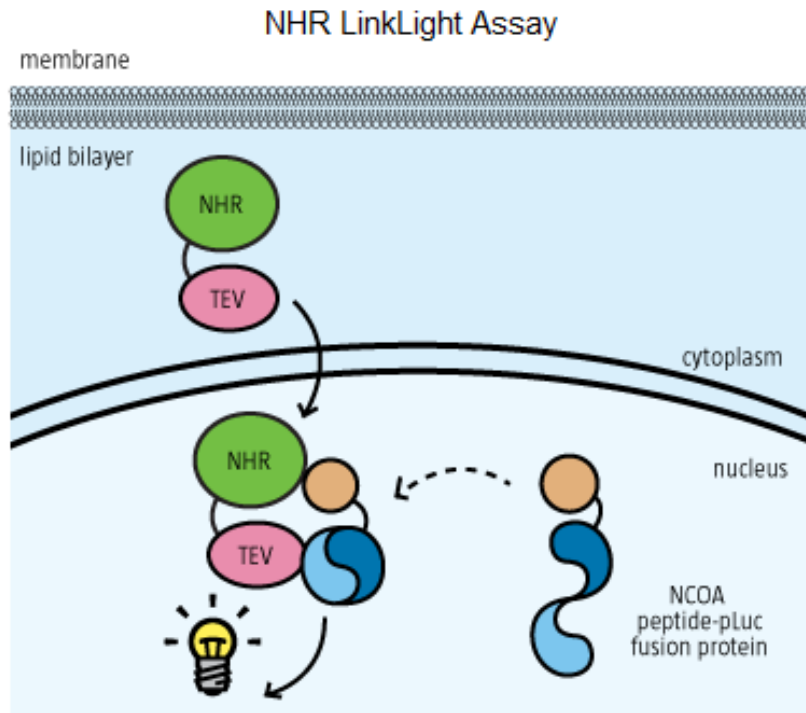
Technology	Advantage	Disadvantage
<p>BRET</p> <p>FRET</p>	Instantaneous, reversible, temporal & specific signal readout	Sensitivity limited by donor-acceptor distance, orientation, spectrum overlap, donor/acceptor ratio, difficult to operate, unable to generate stable cells
<p>EFC & PFC</p> <p>(Enzyme or protein fragment complementation)</p> <p>PathHunter™</p>	Quick signal readout, specific, easy to operate.	Fragment spontaneous self-complementation can result in high background, false interactions.
<p>Tango™</p> <p>(transcriptional based)</p>	sensitive, easy to operate.	Off-target hits due to long transcription/translation process, irreversible
<p>LinkLight™</p>	Sensitive, specific, & quick signal readout, Multiplex ability, easy to operate & low cost of detection reagents.	Irreversible

Current GPCR β -arrestin LinkLight Cell Lines

GPCR β -arrestin-2 LinkLight™ cells	Cat. #	GPCR β -arrestin-2 LinkLight™ cells	Cat. #
GPR1	1001	CXCR7 (CMKOR1)	1027
ADRB2 (b2AR, b2)	1002	HTR2C (5HT2C)	1028
CMKLR1 (ChemR23)	1003	LPAR3 (EDG7)	1029
FFA2 (GPR43)	1004	EDNRB (ETb)	1030
AVPR2 (V2R, V2)	1005	ADORA2B (A2B)	1031
HTR2A (5HT2a)	1006	GPBAR1 (TGR5)	1032
GPR109A (HM74a)	1007	NTSR1 (NT1)	1033
GPR119 (GPCR2)	1008	GPR84 (EX33)	1034
NMUR1 (GPR66)	1009	P2YR4 (P2Y4)	1035
S1P1 (EDG1)	1010	BDKRB1 (B1)	1036
BDKRB2 (B2)	1012	S1PR2 (EDG5)	1037
CCKAR (CCK1)	1013	P2YR12 (P2Y12)	1041
CCKBR (CCK2)	1014	MLNR (GPR38)	1042
GALR1 (GALNR1)	1018	LPAR4 (P2Y9, GPR23)	1043
GLP1R	1019	O3FAR1 (GPR120)	1044
DRD2 (D2)	1020	GPR183 (EBI2)	1045
S1PR3 (EDG3)	1021	FFAR3 (GPR41)	1046
S1PR4 (EDG6)	1022	FFAR1 (GPR40)	1047
S1PR5 (EDG8)	1023	KISS1R (GPR54)	1048
LPAR2 (EDG4)	1024	ADORA1 (A1AR, RDC7)	1049
DRD1 (D1)	1025	GPR55	1050
DRD3 (D3)	1026	CCR6	1051

NHR LinkLight™ Assay

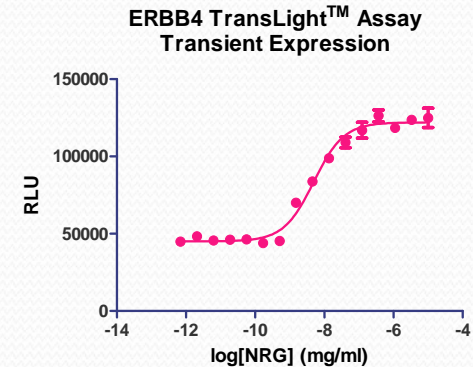
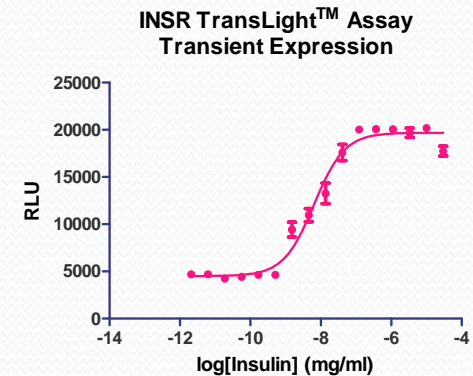
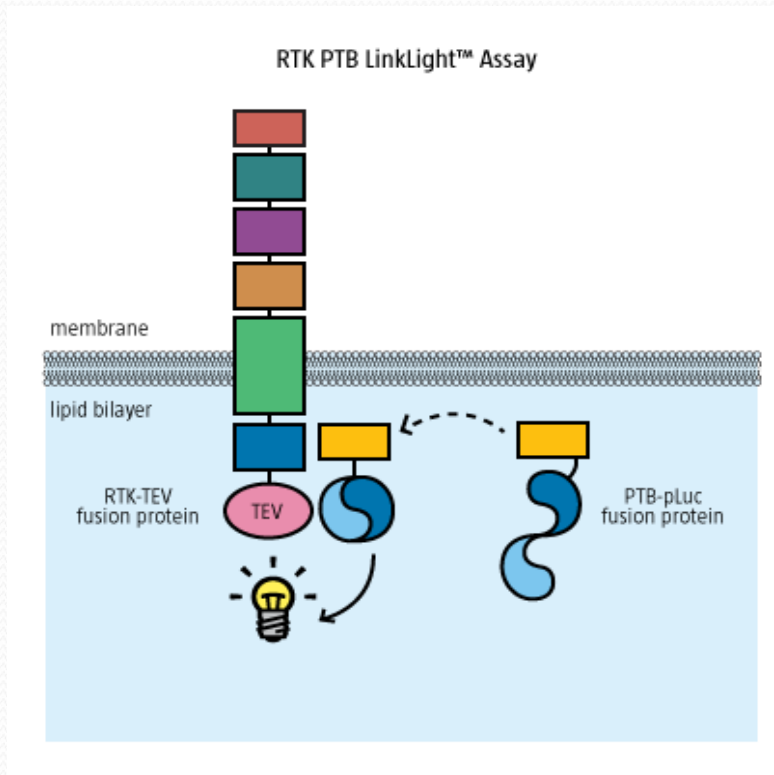
Examples of NHR LinkLight™ Assay



A nuclear hormone receptor (NHR) is linked with the TEV protease. A nuclear receptor co-activator-1 interaction domain (NCOA-1) is linked with a permuted luciferase containing a TEV cleavage sequence. Upon NHR migrating into the nucleus and forming a complex with NCOA-1, the permuted luciferase is cleaved. The cleaved fragment spontaneously refolds to form active luciferase.

RTK LinkLight™ Assay

Examples of RTK LinkLight™ Assay



Upon ligand binding, Receptor Tyrosine Kinases (RTK) interact with a phosphotyrosine binding (PTB) domain protein. The interactions between RTK-TEV and PTB-pLuc result in cleavage of permuted luciferase. The cleaved fragment spontaneously refolds to form active luciferase.

BioInvenu Offers

- GPCR LinkLight™ assay cell lines, transient or division-arrested cells
- GPCR LinkLight™ assay open system including reporter host cell lines and cloning vectors for customers to establish their own target assays
- NHR LinkLight™ assay cell lines, transient or division-arrested cells
- RTK LinkLight™ assay cell lines, transient or division-arrested cells
- Customer assay developments, compound profiling, & screening services.

LinkLight™ assay detection reagent is a fraction of other assay's cost!



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Thank You!

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