Validated Cell-Based Assays for Rapid Screening and Functional Characterization of Therapeutic Monoclonal Antibodies

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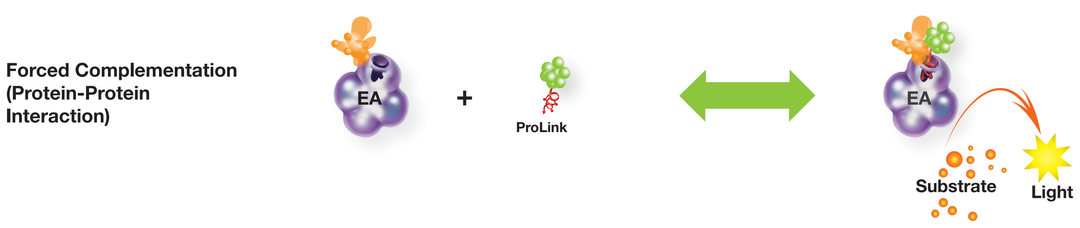
Abstract

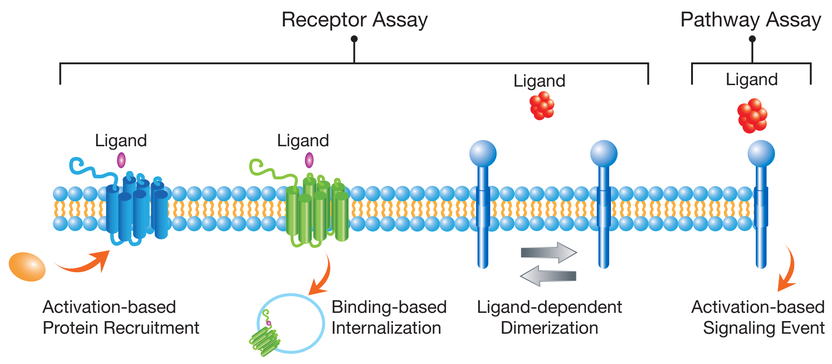
Monoclonal antibodies (MAbs) represent one of the fastest growing classes of biotherapeutic molecules today. While many technologies exist for generating and panning large collections of antibody fragments, there is a clear need for tools that can reliably detect both the binding and functional activity of a monoclonal antibody in a fast, reproducible and cost effective manner. This requires validated and sensitive cell-based assays that provide a target-specific response and have a high tolerance for serum, cellular and bacterial extracts.

Here, we describe the development, validation and application of PathHunter® target-specific cell-based assays that enable the identification and characterization of therapeutic MAbs targeting G-Protein Coupled Receptors, Receptor Tyrosine Kinases, other novel receptor targets and their extracellular ligands. This technology relies on cells expressing full length, native receptors to create assays that are sensitive, scalable, robust and have a simple mix-and-read protocol, which facilitate the detection of antibodies in even the most complex biological samples. This technology has also been adapted to study receptor internalization and receptor dimerization - both being important mechanisms for antibody-induced receptor inhibition. Importantly, these assays have been extensively utilized, not only to discover and develop therapeutic antibodies, but also in the manufacturing QC process and for immunogenicity testing for neutralizing anti-drug antibodies. Relevant validation and application data are presented and discussed.

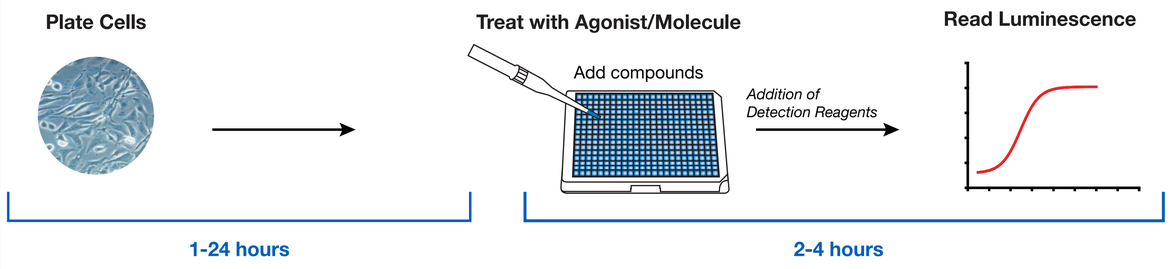
DiscoveRx EFC Technology



Application to Multiple Target Classes



PathHunter β-Arrestin GPCR Assay System



Unmodified Extracellular Domains

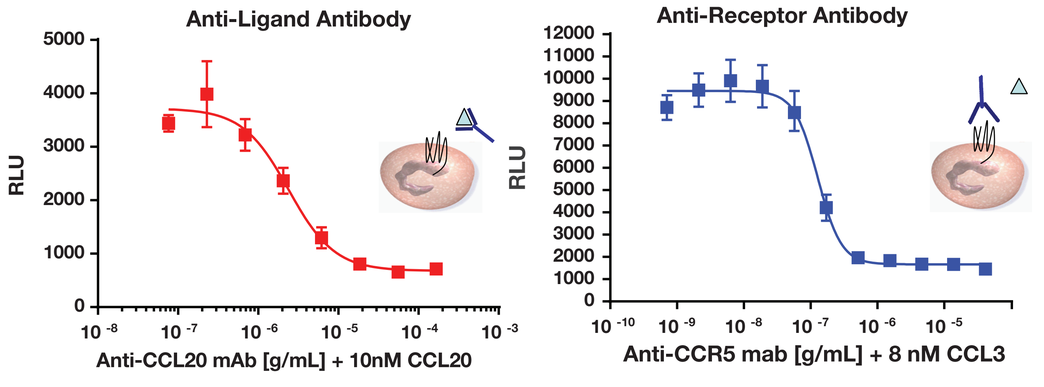
Naked CHO-K1 cells

CHO-K1 CXCR3 cells

With CXCR3-PE Antibody

Since the ProLink tag is on the C-terminus of the GPCR and receptor tyrosine kinase targets, the extracellular domain is unencumbered by any extraneous sequence. For this reason PathHunter cell lines are an ideal solution for the generation of antibodies or panning selected antibodies. In this example and anti-CXCR3 antibody was used to detect expression of CXCR3 at the cell surface. Cells were stained with 1ug/ml of the anti-CXCR3 PE conjugated antibody and analyzed by flow cytometry.

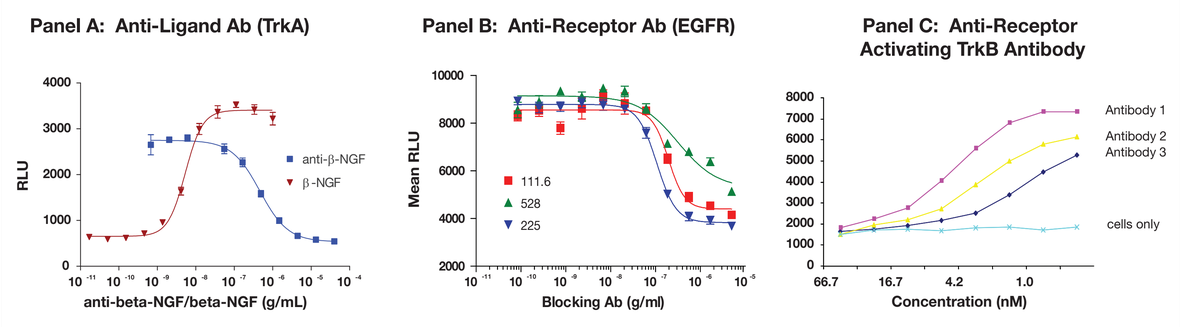
Functional Antibody Characterization: Case Study GPCRs



Antibodies that neutralize the ligand or receptor can be detected.

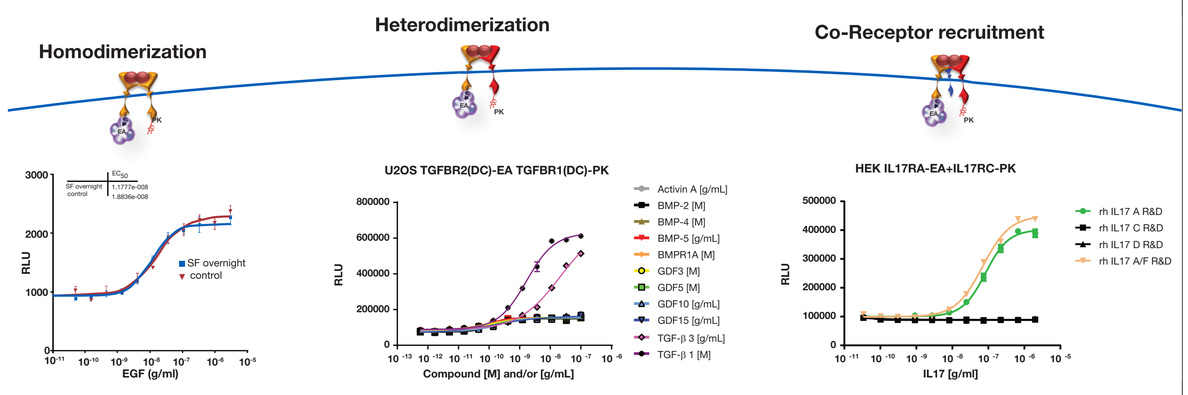
First antibody was added to PathHunter Arrestin cells expressing CCR6. Next the cells were challenged with an EC80 of agonist. Increasing concentrations of antibody are shown to neutralize the ligand and prevent its activation of the target. In the right panel, CCR5 cells were pre-incubated with an anti-CCR5 blocking antibody. Challenge with the EC80 of agonist shows that increasing concentrations of antibody are able to effectively block receptor activation.

Anti-ligand & Anti-Receptor Antibodies



Panel A: The ligand for TrkA is β-NGF. In this experiment, PathHunter TrkA cells were treated with an anti-β-NGF antibody prior to addition of β-NGF. This experiment demonstrates that the PathHunter assay format is capable of detecting activity of an antibody against the ligand for a receptor. **Panel B:** In this experiment PathHunter EGFR cells were used to screen commercially available antibodies that have the ability to block EGFR. The experiment was performed by incubating PathHunter cells with increasing amounts of the blocking antibodies to determine which antibody had the greatest inhibitory effect. In Panel C, antibodies targeting the receptor were panned for their ability to activate the receptor via dimerization. Several activating antibodies were isolated.

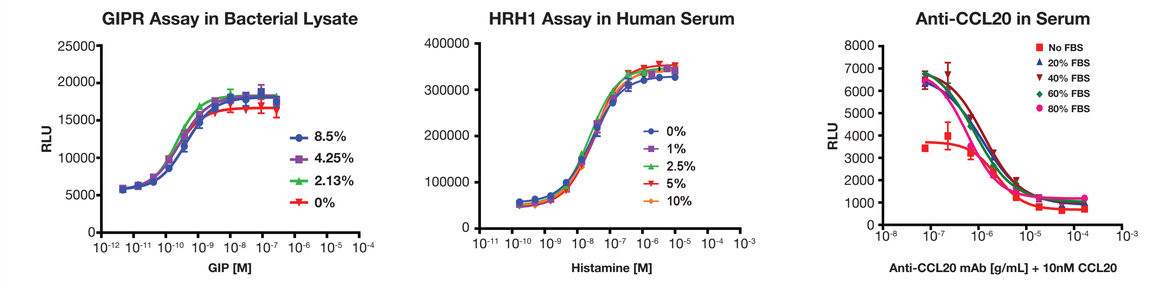
Emerging Technology - Receptor Dimerization Events Monitored at the Cell Surface



Receptor Dimerization events monitored at the cell surface

Historically receptor interactions have been difficult to monitor directly due to the limitations of existing protein interaction technologies. The EFC system overcomes many of these hurdles enabling discovery directly at the target receptor. In this system one receptor subunit is fused to EA and the second is fused to PK. The system can be used to look at simple homodimerization events such as the EGFR, or heterodimerization events such as the TGF-β receptors (center panel) or more complex stochiometries such as IL-17 heterodimerization (right panel).

Compatible with High Serum Levels & Neat Blood Plasma



Target proximal assays are resistant to serum effects.

PathHunter assays can detect functional biologics in various samples, such as human serum, plasma, hybridoma lysates and bacterial lysates. Above, we validated with GIPR assay with up to 8.5% bacterial lysate, the HRH1 assay with up to 10% normal human serum and were able to detect antibody-based inhibition of the CCL20 ligand in up to 80% serum without any loss of sensitivity.

Arrestin as a Generic Tool

A subset of the vast number of assays available for biologics development.

>600 GPCR Assays

Assays for Multiple GPCR Families			
Acetylcholine	CRHR1&2	MCHR1&2	Prokineticin
Adenosine	Dopamine	Melanocortin	PRLHR
Adrenoreceptor	Endothelin	Melatonin	Prostanoid
Anaphylotoxin	Estrogen	GRM	Protease
Angiotensin	Formylpeptide	Motilin	PTH receptor
Apelin	Free Fatty Acid	NMUR1&2	Relaxin
Bile Acid	GABBR1&2	NPBWR1&2	Serotonin
Bombesin	Galanin	NPFFR1&2	Somatostatin
Bradykinin	Ghrelin	NPSR1	Succinate
Calcitonin	Glucagon	Neuropeptide Y	Tachykinin
Calcium-Sensing	Glutamate	Neurotensin	TAAR1
Cannabinoid	Glycoprotein H	Opioid	TRHR
Chemerin	GNRHR	Orexin	UTS2R
Chemokine	5HT Receptors	Orphans	Vasopressin
Cholecystokinin	Histamine	Orthologs	VIP & PACAP
Class A Orphan	HCA Receptors	OXGR1	
Class B Orphan	KISS1R	P2Y	
Complement	Leukotriene	QRFPR	
Corticotropin	LPA & S1P	PTAFR	

>30 RTK Assays

RTK Assays	
c-KIT	ErbB4
c-MET	FGFR1
c-Ret-GFRα2	IGF1R
DDR1	INSR
DDR2	INSR (Internalization)
EphA4	PDGFRa
EphA5	PDGFRb
EphA7	TrkA
EphB1	TrkA-P75
EphB2	TrkB
EphB3	TrkB-P75
EphB4	TrkC
ErbB1	TrkC-P75
ErbB2-ErbB3	

Receptor Assays
TNF receptor
TGFbR1&2
BMP receptors
TLR receptors
IL17 receptors
Frizzled receptor

Cytokine Receptors
GCSF Receptor
Epo Receptor
GHR
PRLR

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