



Accelerate Your COVID-19 Drug Discovery with Qualified Cell-Based Assays for Proinflammatory Cytokines

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Scientific Development Manager, Eurofins DiscoverX

OUR EXPERTISE
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DISCOVER
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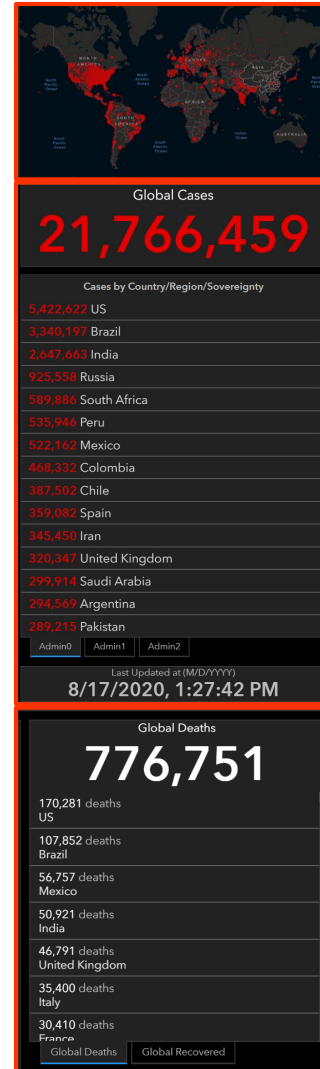
COVID-19: Therapeutic Approaches for Disease Management

The pathophysiology of COVID-19 in severe cases shows an aggressive inflammatory responses. Thus the disease severity is not only due to the viral infection but also the host's *immune response to the infection*.

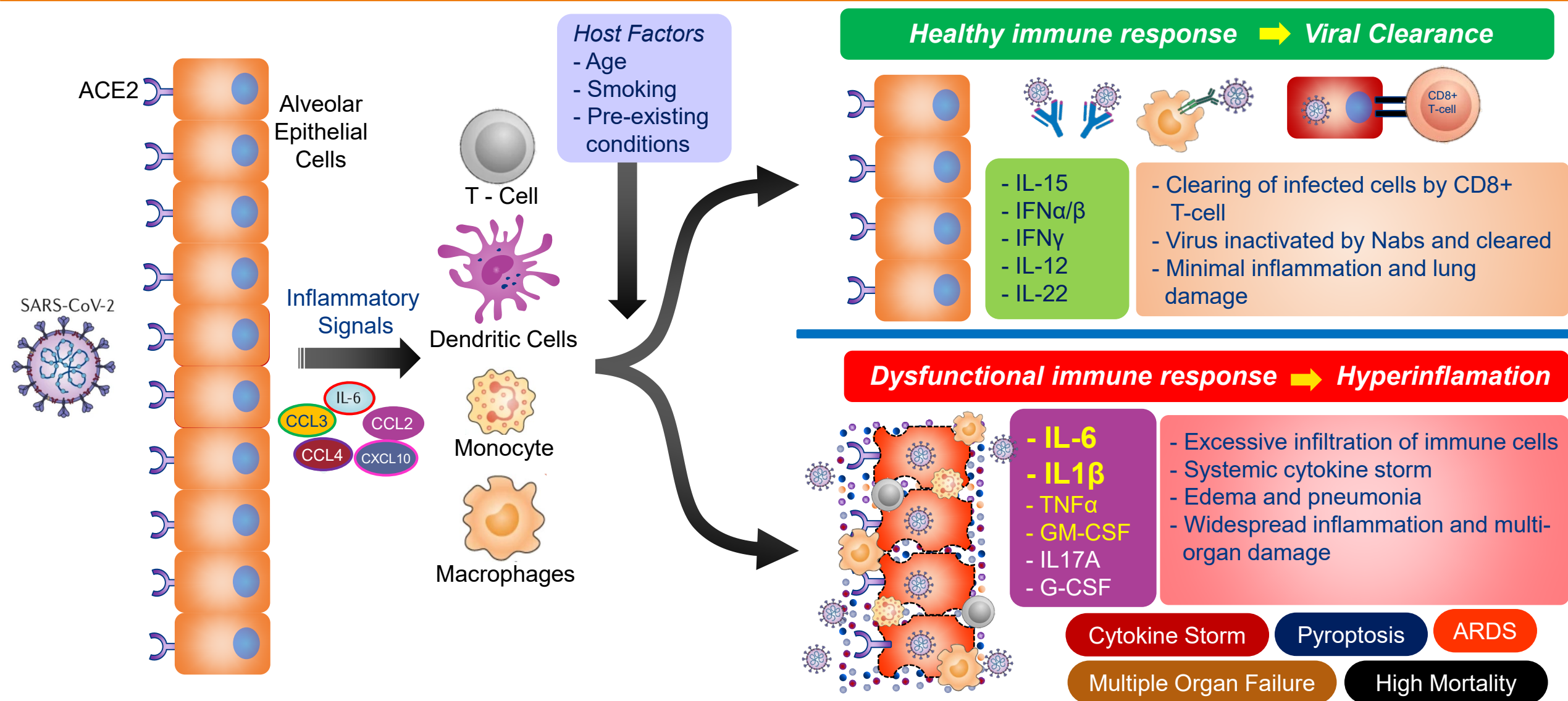
Therefore alongside investigations into the virology of SARS-CoV-2, it is also imperative to build an understanding into the fundamental *immunological* processes underlying the clinical manifestations of COVID-19 disease for identification and a rational design of effective therapies.

Cytokine release syndrome (CRS), commonly referred to as “*cytokine storm*” is the major cause of death in fatal COVID-19 cases. It is caused by a dysfunctional hyper-release of proinflammatory cytokines by the host immune system in response to the viral infection and/or secondary infections, which causes an uncontrolled inflammation leading to multi-organ failure.

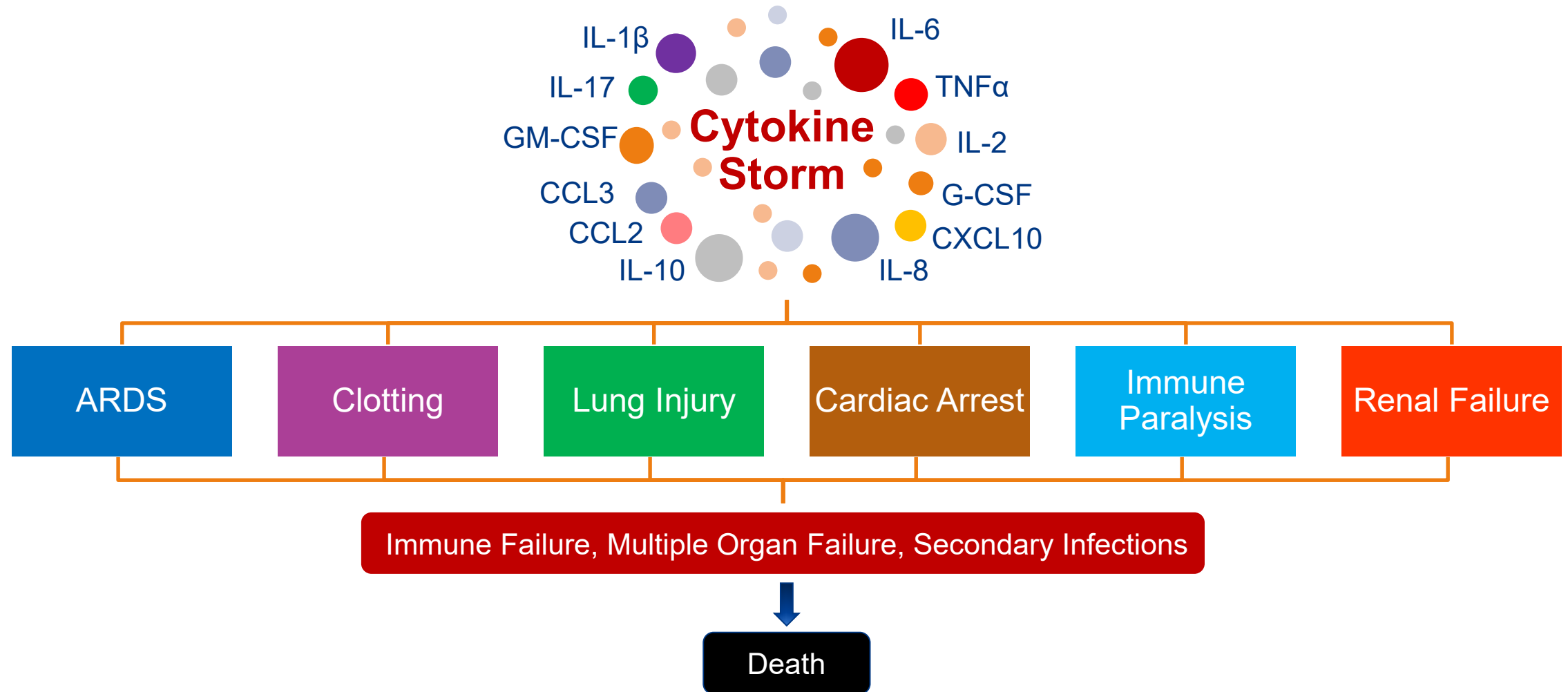
Acute respiratory distress syndrome (ARDS) can lead directly to respiratory failure is closely related with cytokine storm. Proinflammatory cytokine levels are significantly elevated in patients with ARDS, and the degree of increase is positively correlated with mortality rate.



Proinflammatory Cytokine Pathogenesis of COVID-19



Implications of Cytokine Storm in COVID-19 Patients



Withstanding the Cytokine Storm in COVID-19: Therapeutic Approach

Cytokine Storm



 *Eurofins DiscoverX Cell-Based Functional Assay Available!*

Analytical Assays for Therapeutics Targeting Key Proinflammatory Cytokines

1. Qualified Bioassays:

- Case Study with PathHunter® Tocilizumab Bioassay - Qualified with  **ACTEMRA®** *tocilizumab* for therapeutics targeting IL-6 pathway
- IL-1 β - PathHunter® Anakinra Bioassay - Qualified with  **Kineret®** (anakinra)
- GM-CSF - PathHunter® Sargramostim Bioassay – Qualified with **Leukine®** *sargramostim*
A Recombinant GM-CSF–Yeast-Expressed
- TNF α - PathHunter® Adalimumab Bioassay - Qualified with **HUMIRA** *adalimumab*

2. Signaling Reporter Assays

- IL-6
- TNF α
- RANK (TNFSF11)

3. Functional Cell-Based Assays for Small Molecule Inhibitors

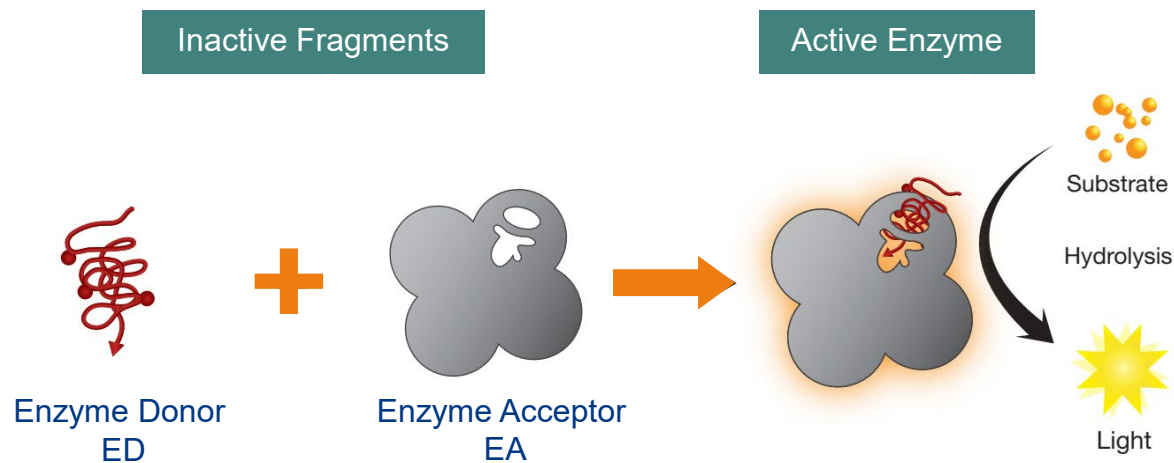
- JAK1, JAK2 and JAK3

Technology Overview

Enzyme Fragment Complementation



Enabling Technologies with a Flexible Platform based on a Split β -Galactosidase Enzyme



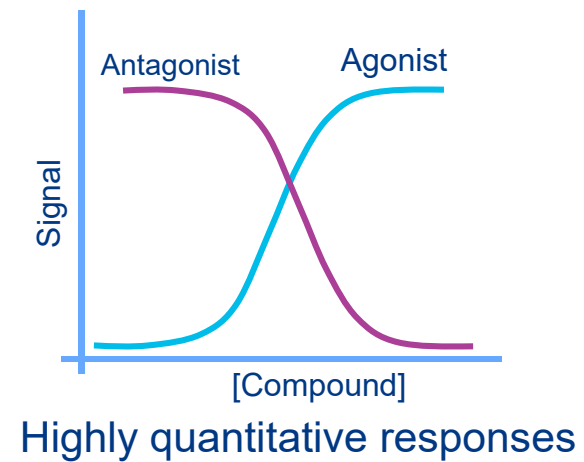
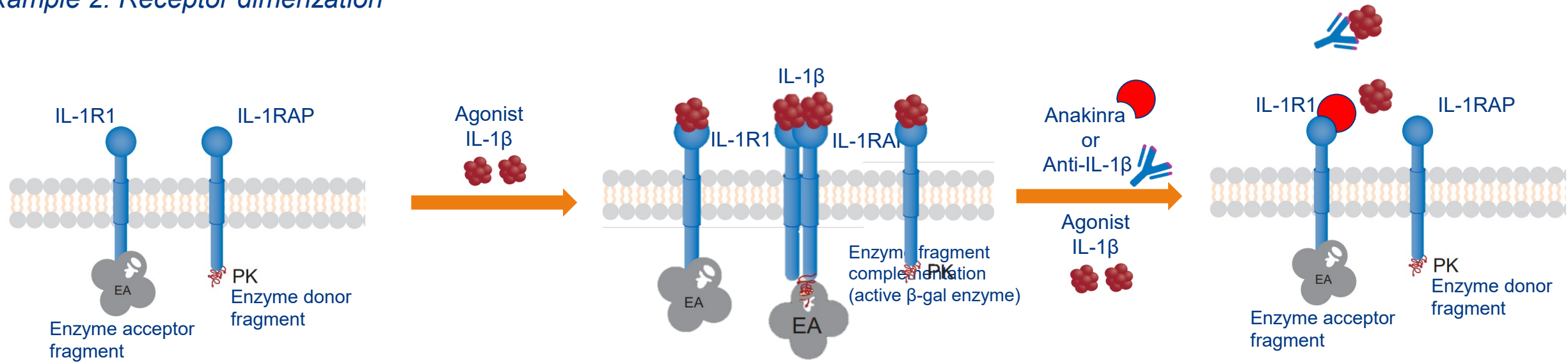
EFC Assay Principle:

- Complementation between two inactive enzyme fragments called the Enzyme Donor (ED) and Enzyme acceptor (EA) results in formation of an active β -gal enzyme.
- The active functional β -gal enzyme hydrolyzes its substrate to produce chemiluminescent signal.

- **Homogenous Format** — Mix-and-read assay format that does not require washing, centrifugation, or filtration
- **Robust** — Enzymatically-amplified assays with a large signal-to-background ratio and high precision with Z' factors >0.7 and lot-to-lot reproducibility
- **Qualified and Validated** — Extensively optimized for hundreds of targets used for screening in billions of data points, and thousands of peer-reviewed publications
- **Scalable** — Easily scalable and HTS-friendly from 96- to 1536-well microplate format

Enzyme Fragment Complementation (EFC) Versatile and Robust Platform for Cell-Based assays

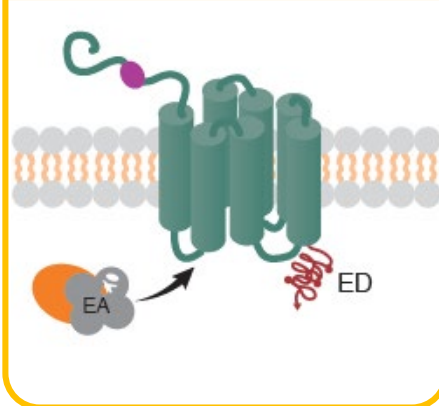
Example 2: Receptor dimerization



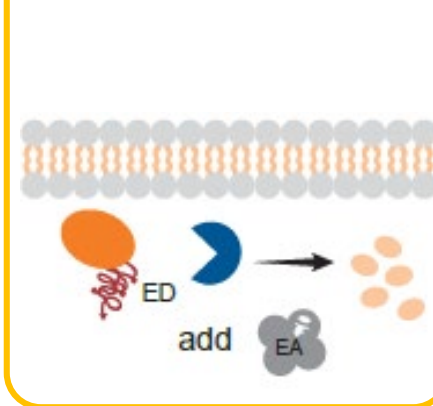
Enzyme Fragment Complementation (EFC)

Versatile and Robust Platform for Cell-Based Assays

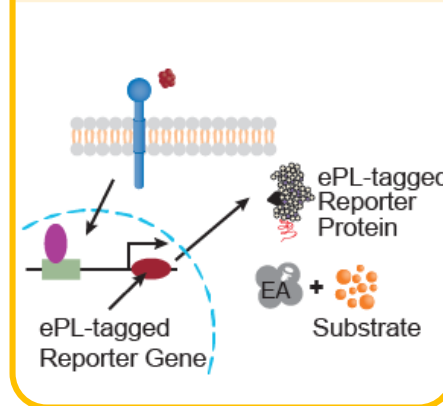
Protein-Protein Interaction



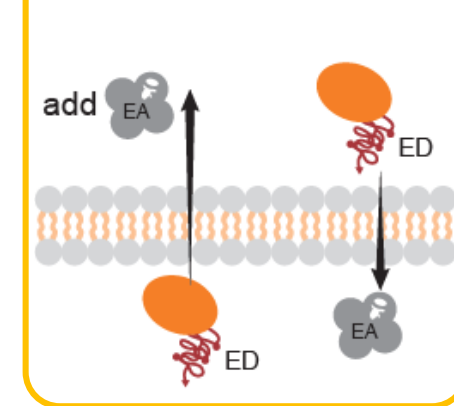
Quantitation and Degradation



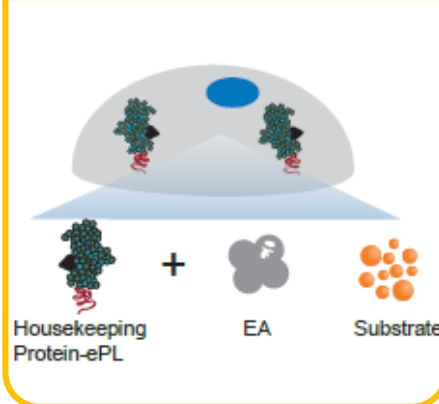
Downstream Response Evaluation



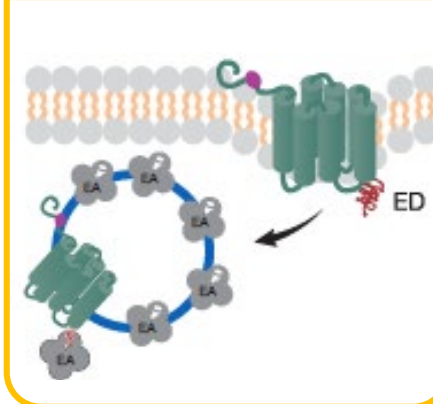
Secretion and Translocation



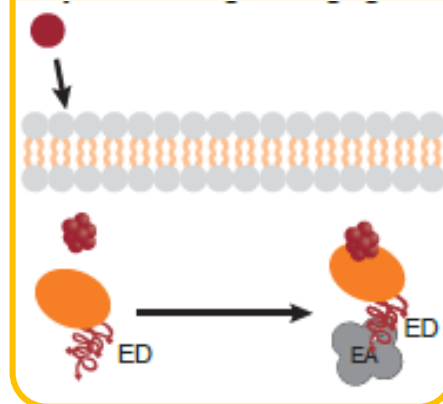
Quantify Cytotoxicity



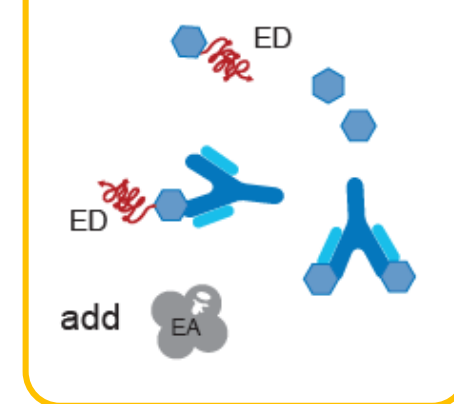
Internalization and Trafficking



Compound Target Engagement



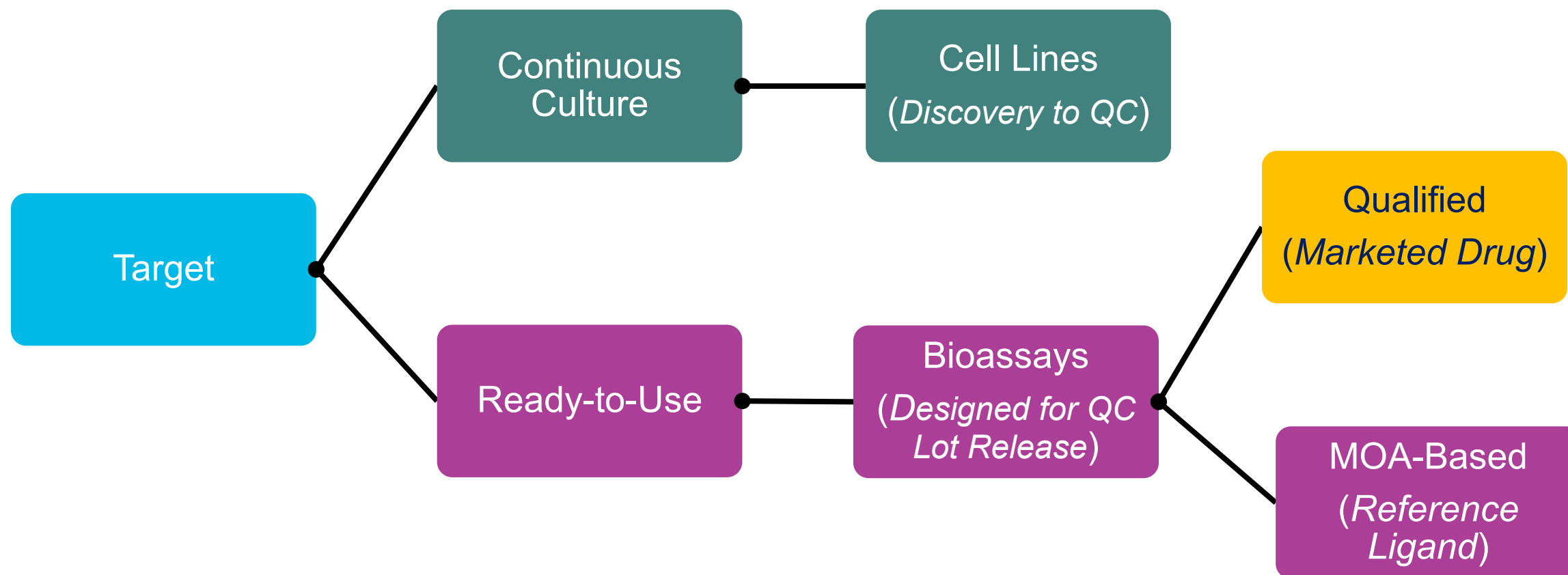
Analyte Detection



Flexibility of Formats

Phase-Appropriate Solutions for Every Development Program

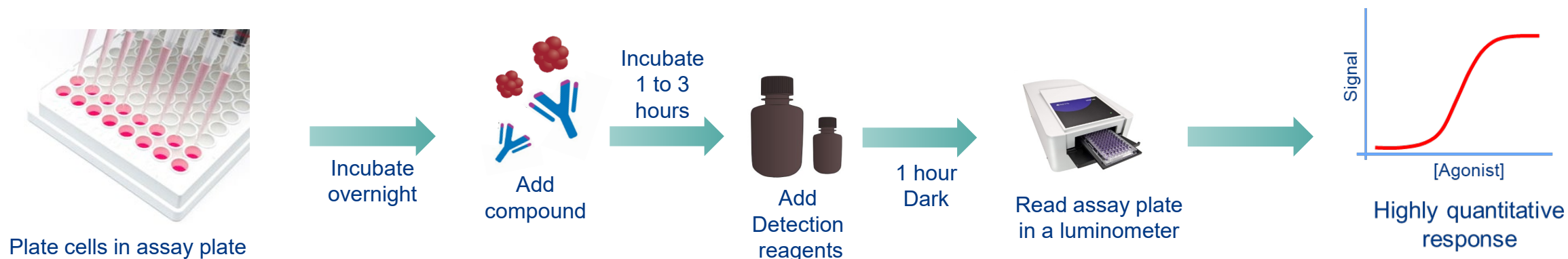
From Continuous Culture to Qualified Bioassays



Eurofins DiscoverX Qualified Bioassays are designed for QC Lot Release Programs

EFC Cell-Based Assays Protocol

Typical assay protocol for stable cell lines and assay ready kits



Simplified, universal assay format

- Easy-to-follow, detailed user manuals
- Rapid, single addition protocol
- Homogeneous, no wash format

Sensitive detection with large S:B ratio

Easily quantified luminescence read-out

- Dose-response curve
- Utilizes any standard plate luminometer

Everything is included!



Bioassay Kit includes all reagents needed for the assay. No need to worry about external sourcing of media reagents that may introduce variability

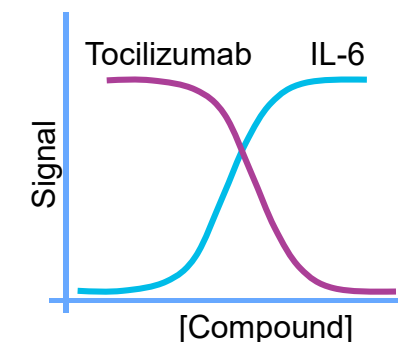
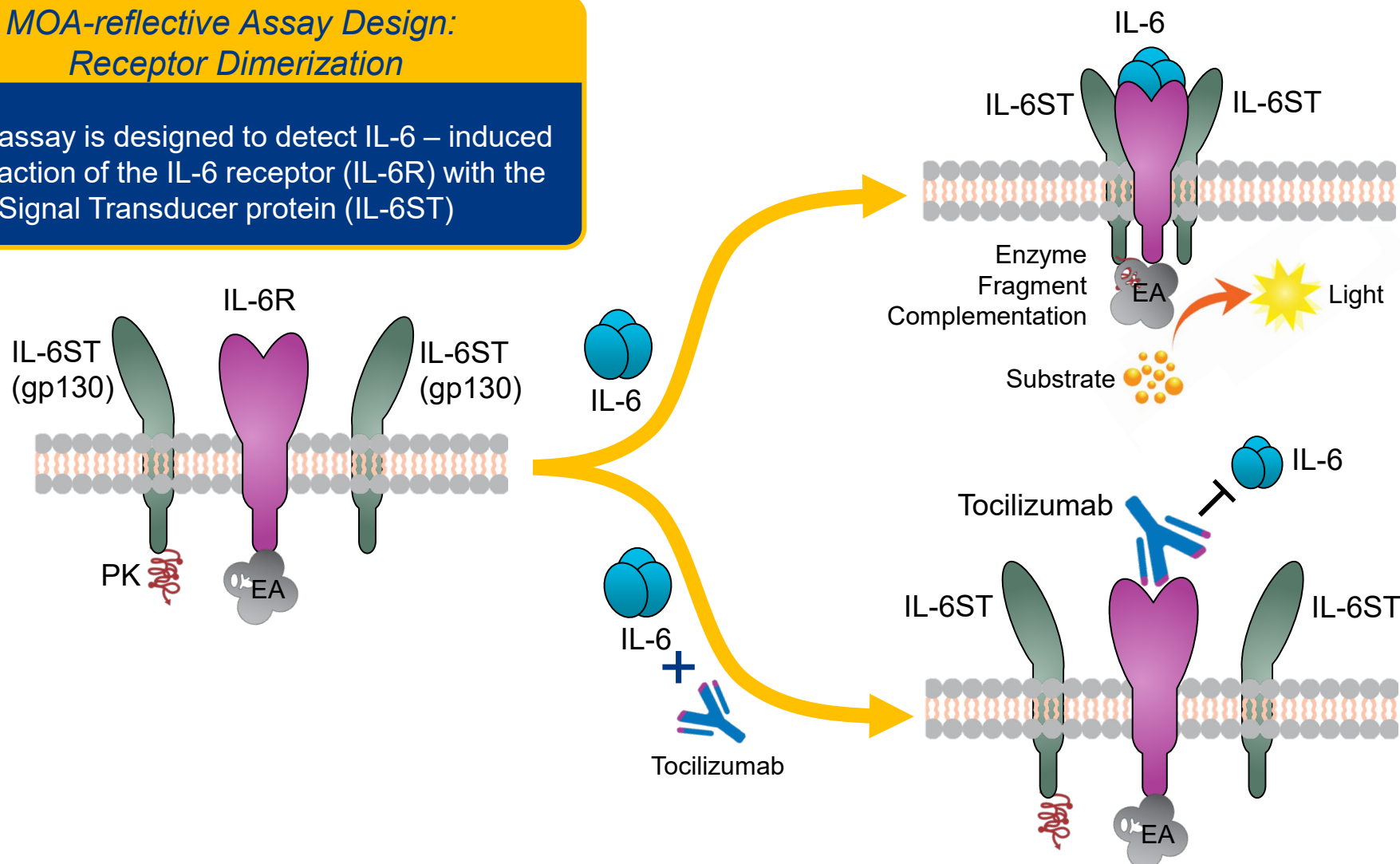
Case Study:

PathHunter[®] Tocilizumab Bioassay - Qualified with Actemra[®] for Therapeutics Targeting IL-6 Activity

PathHunter® Tocilizumab Bioassay - Qualified with Actemra®

MOA-reflective Assay Design: Receptor Dimerization

The assay is designed to detect IL-6 – induced interaction of the IL-6 receptor (IL-6R) with the IL-6 Signal Transducer protein (IL-6ST)



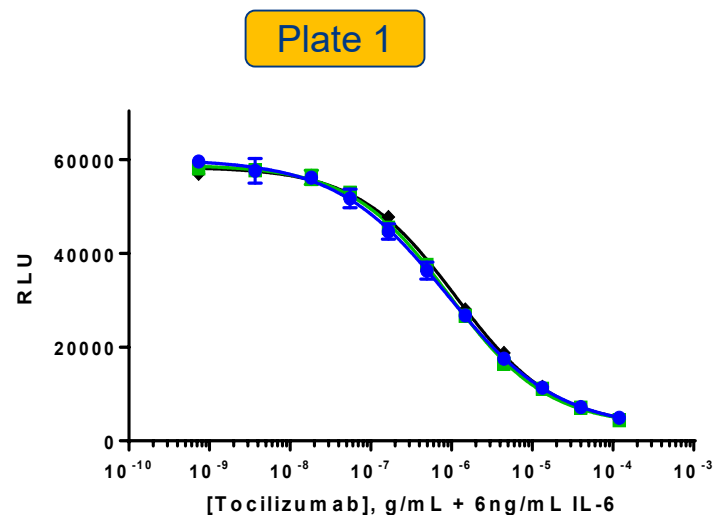
Actemra® is a registered trademark of Chugai Seiyaku Kabushiki Kaisha Corp., a member of the Roche Group

- Assay consistency (%CV) between eight 11-pt DRCs
- Plate uniformity: EC_{80} and IC_{80} (of IL-6 and Tocilizumab) across entire plate
- Plate-to-Plate variability: 3 plates with 11-pt DRCs run on 3 days
- Slope consistency
- Relative potency analysis: Accuracy, precision and linearity of the assay over a range of 50-150% performed by two operators
- Parallel line analysis using PLA3.0 or SoftMax Pro 7.1

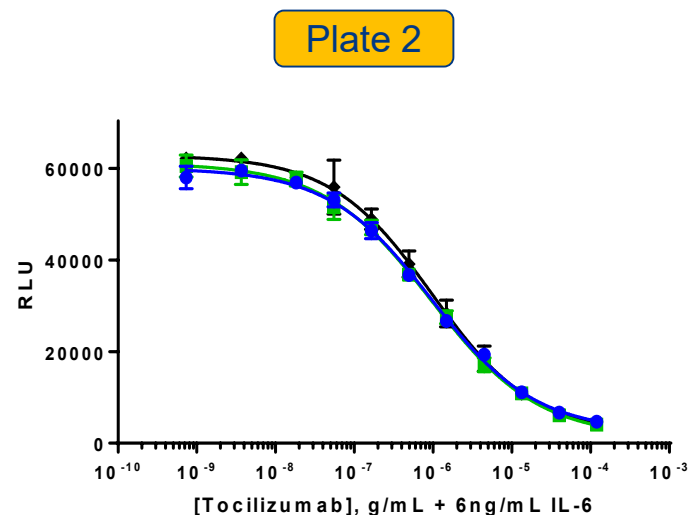


Assessing Intra-Day Plate-to-Plate Variability

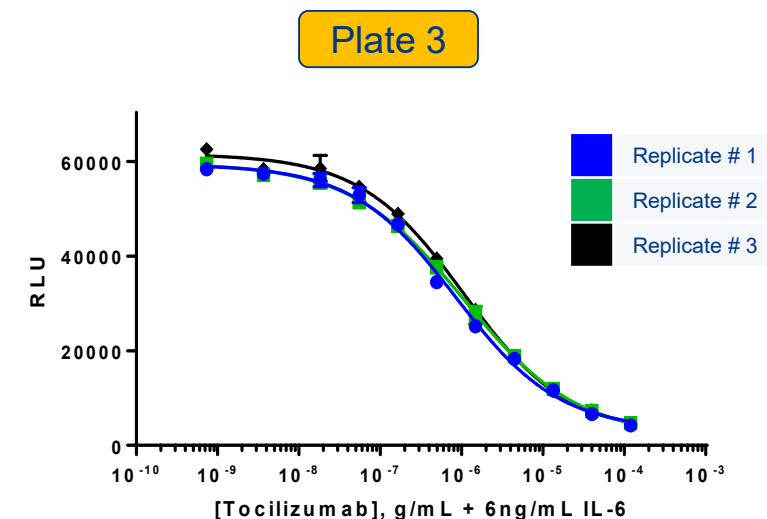
Experiments depicting 3 assay plates with full-plate DRC - Same Day – One operator



Parameter	R1	R2	R3
S/B	12.1	13.7	12.6
Hill Slope	-0.6404	-0.7106	-0.7084
IC ₅₀ (ng/mL)	868	935	1,131



Parameter	R1	R2	R3
S/B	13.2	15.2	14.1
Hill Slope	-0.6622	-0.6366	-0.6781
IC ₅₀ (ng/mL)	966	943	972



Parameter	R1	R2	R3
S/B	14.5	12.8	13.8
Hill Slope	-0.683	-0.624	-0.6799
IC ₅₀ (ng/mL)	850	1077	1063

Repeatability and Intermediate Precision (Inter-Plate)

Plate	Sample	S/B	% RSD, S/B	IC ₅₀ , ng/mL	Mean IC ₅₀ , ng/mL	% RSD, IC ₅₀
1	R1	12.1	6.4	868	978	13.9
	R2	13.7		935		
	R3	12.6		1130		
2	R1	13.2	7.1	966	960	1.59
	R2	15.2		943		
	R3	14.1		972		
3	R1	14.5	6.2	850	997	12.8
	R2	12.8		1080		
	R3	13.8		1060		

Intermediate Precision (Inter-Day)

Day	IC ₅₀ , ng/mL	Mean IC ₅₀ , ng/mL	%RSD, IC ₅₀
1	1250	1330	24.6
2	1690		
3	1050		

Intermediate Precision (Inter-Plate): 1.89%

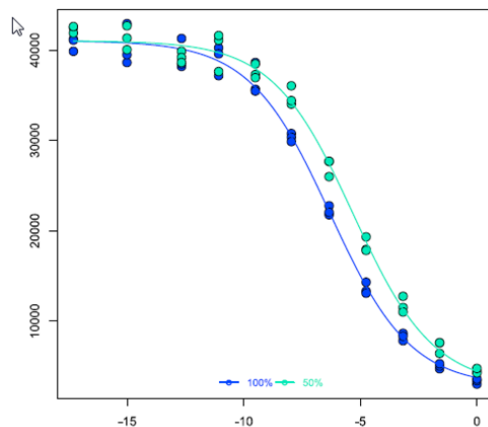
Intermediate Precision (Inter-Day): 24.6%

Parallelism and Relative Potency Estimation

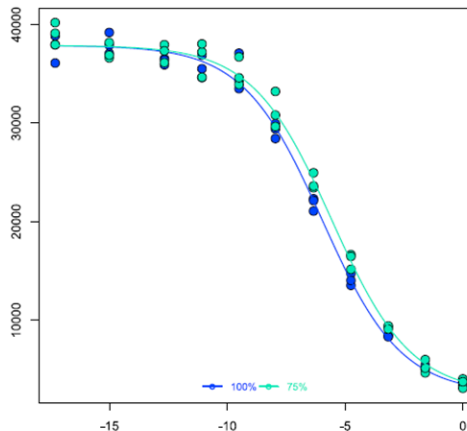
Relative Potency: Parallelism and Potency Estimation (PLA)

Analyst 1

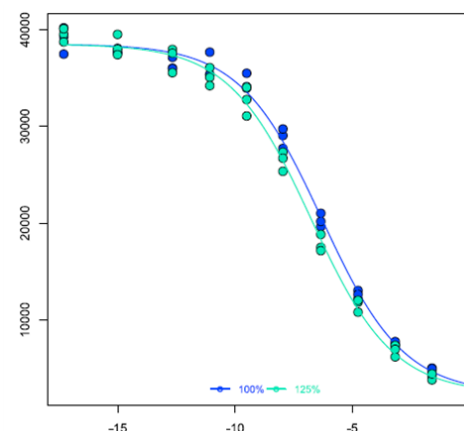
50%



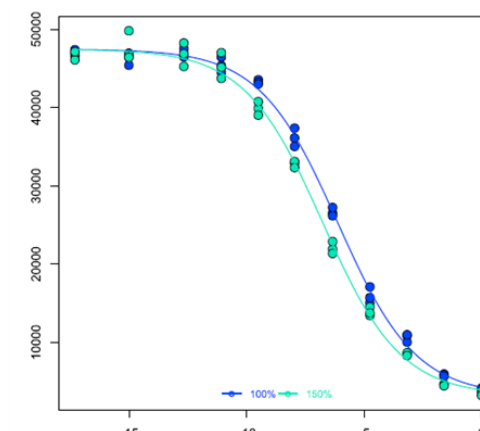
75%



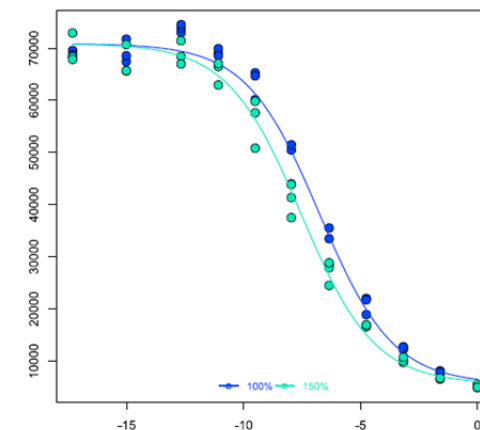
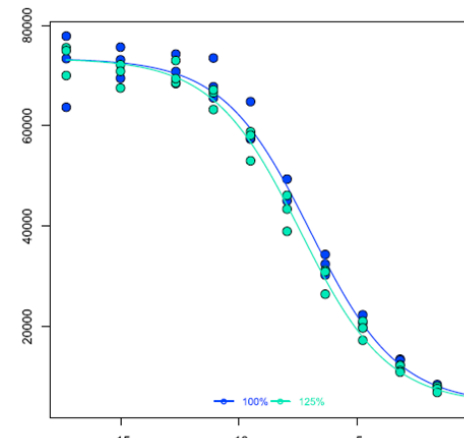
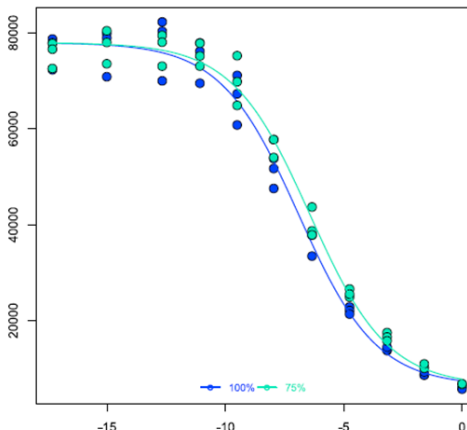
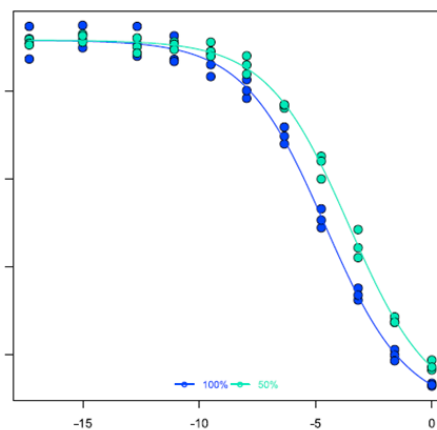
125%



150%



Analyst 2



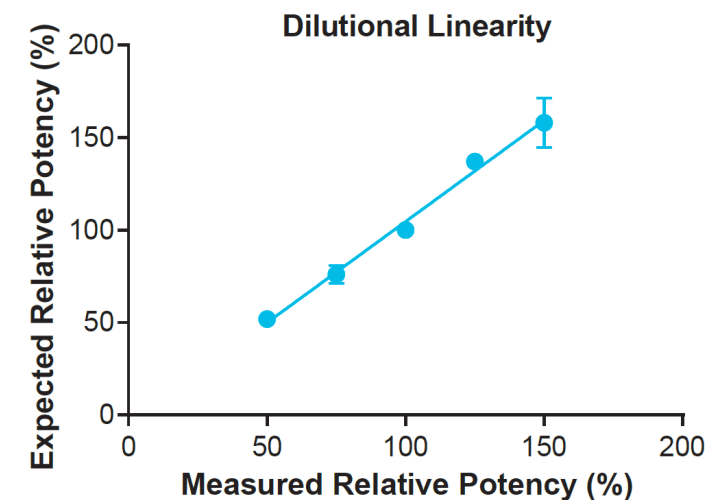
● Reference
● Sample

Note: RP values calculated in PLA 3.0

Tocilizumab Bioassay: Qualification with Actemra®

Summary: Accuracy, Precision and Dilutional Linearity (Single Analyst)

Experiment #	Analyst #	Expected RP (%)	Observed RP (%)	Average RP (%)	% RSD	% Recovery
1	1	150	155.5	158.1	8.44	105.4
2	1		163.2			
3	1		141.1			
4	2		172.7			
1	1	125	138.6	137.1	1.66	109.7
2	1		137.4			
3	1		138.6			
4	2		133.8			
1	1	75	74.1	76.1	6.34	101.5
2	1		83			
3	1		75.4			
4	2		71.9			
1	1	50	54.8	52	4.96	103.9
2	1		50.6			
3	1		53.3			
4	2		49.1			



Dilutional Linearity: 99.27%

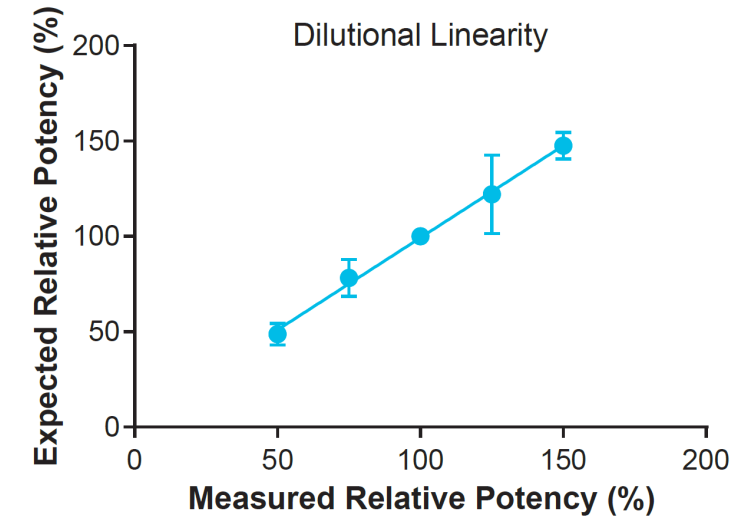
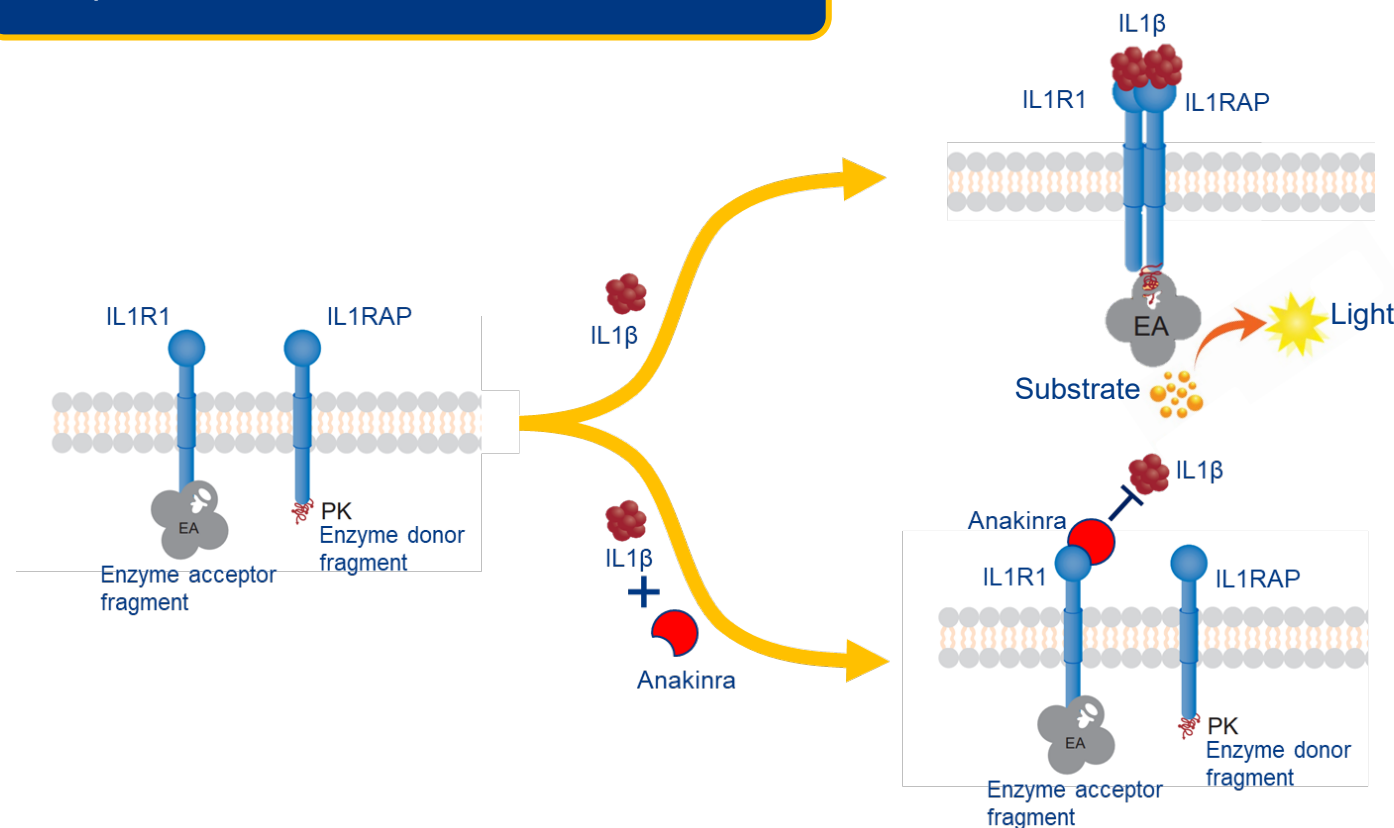
Accuracy: 105.1%

Precision: 5.4%

Case Study 2: IL-1 β PathHunter[®] Anakinra Bioassay - Qualified with Kineret[®]

Assay Design : Receptor Dimerization

The assay is designed to detect IL-1 β induced heterodimerization of the IL1R1 and IL1RAP receptors



Dilutional Linearity: 96.7%

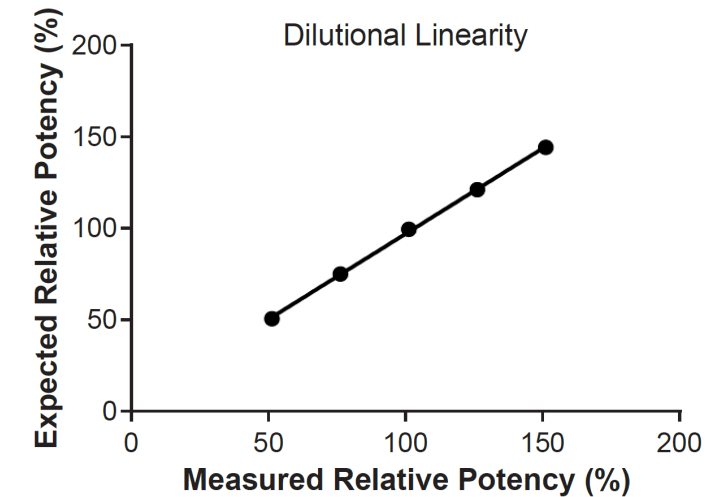
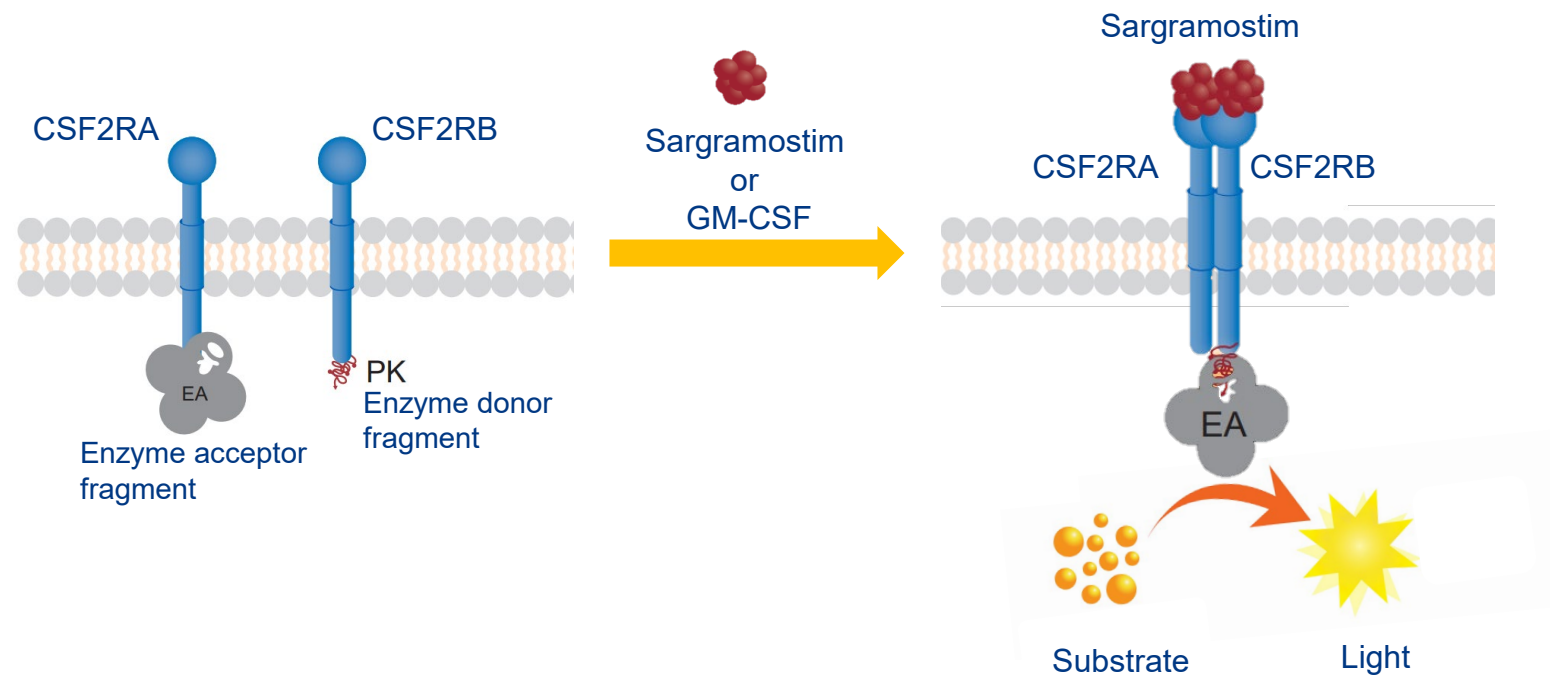
Accuracy: 99.4%

Precision: 11.4%

Case Study 4: GM-CSF PathHunter[®] Anakinra Bioassay - Qualified with Leukin[®]

Assay Design : Receptor Dimerization

The assay is designed to detect GM-CSF-induced heterodimerization of the CSF2RA and CSF2RB receptors



Dilutional Linearity: 99.56%

Accuracy: 99.1%

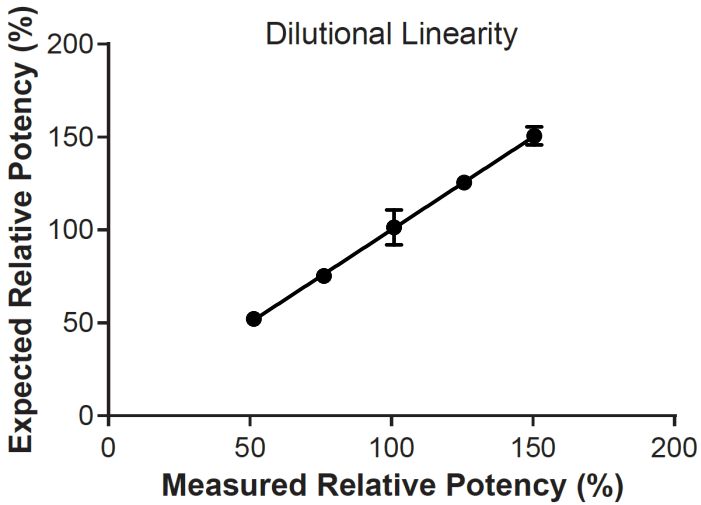
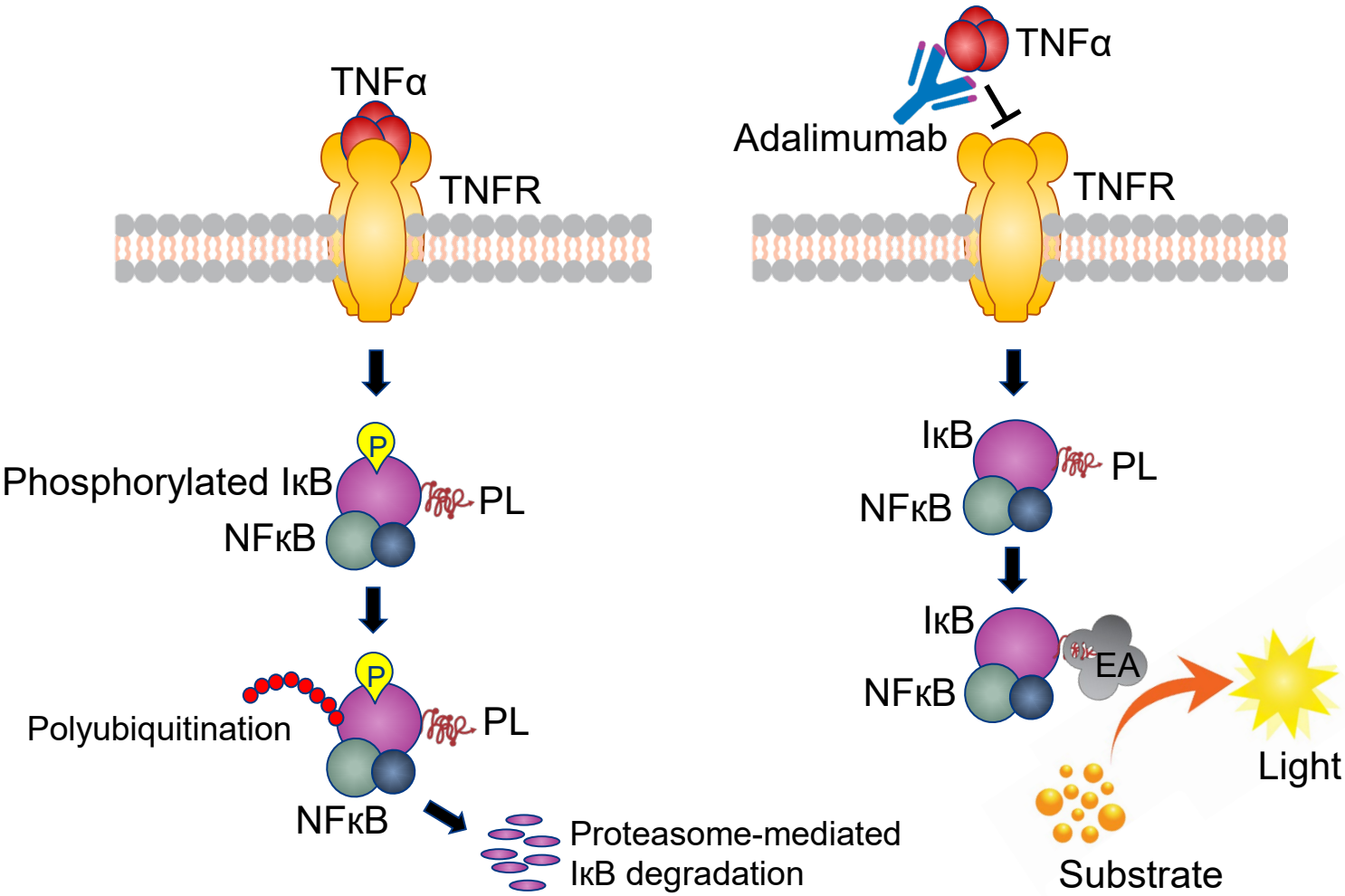
Precision: 3%

Leukine[®] is a registered trademark licensed to Genzyme Corporation

Case Study 3: TNFα PathHunter® Adalimumab Bioassay - Qualified with Humira®

Assay Design : Protein Degradation

The assay is designed to measure IκB degradation as a result of TNFα-mediated activation of the NF-κB signaling pathway.



Dilutional Linearity: 99.99%

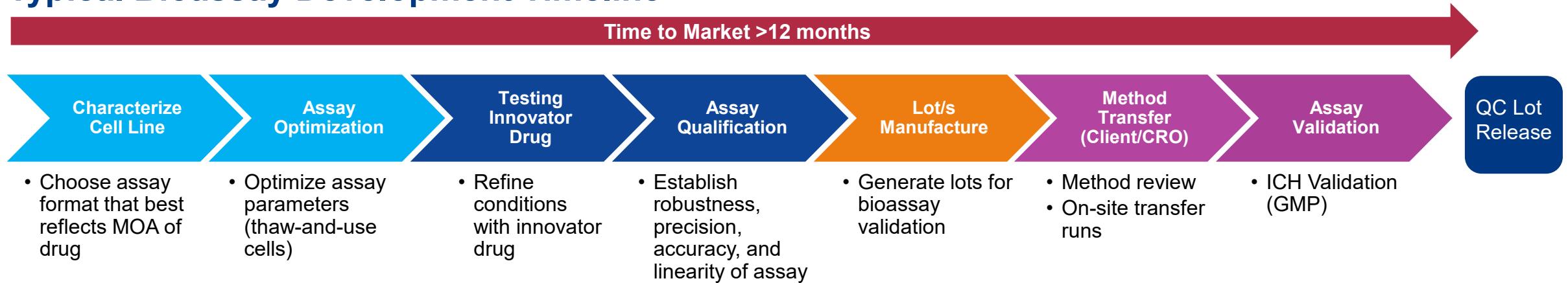
Accuracy: 105.1%

Precision: 5.65%

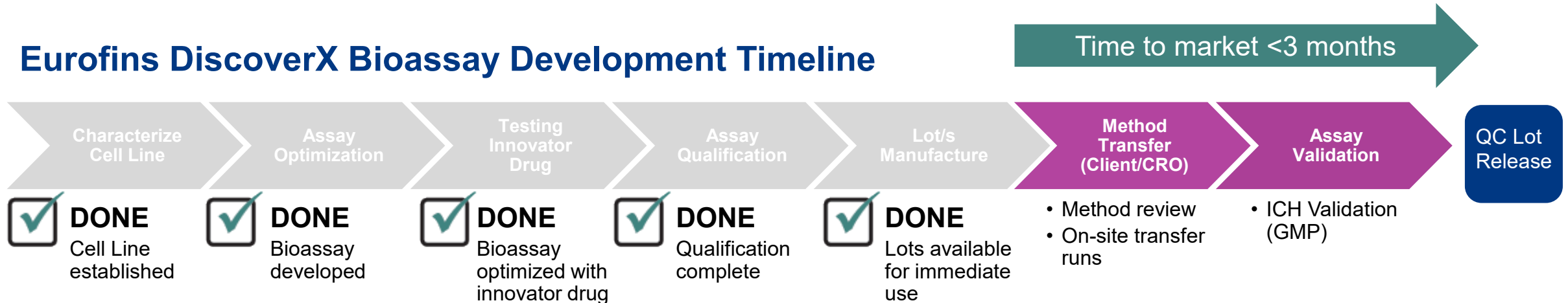
Humira® is a registered trademark AbbVie, Inc

Accelerating Implementation Phase for QC Lot Release with Qualified Bioassays

Typical Bioassay Development Timeline



Eurofins DiscoverX Bioassay Development Timeline

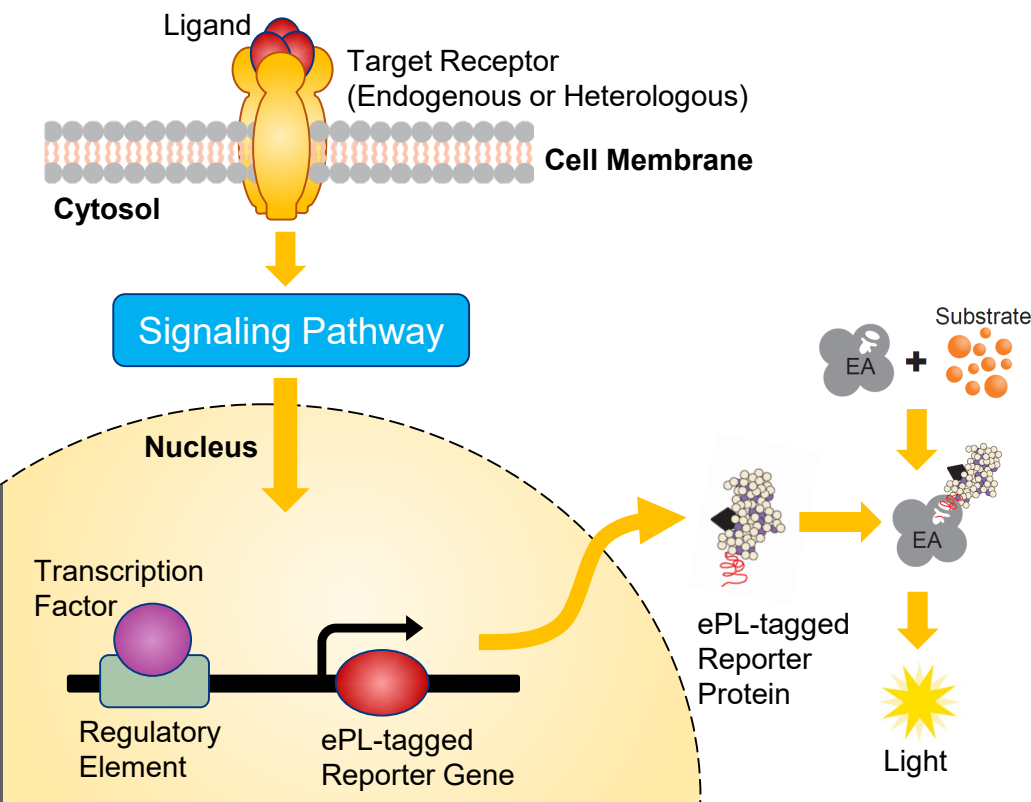


Eurofins DiscoverX Ready-To-Plate Bioassay Kits Save 9 Months of Assay Development Time

Signaling Pathway Reporter Assays for Cytokines

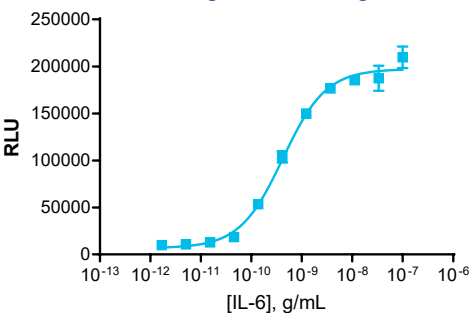
Assay Design : Signaling Reporter

The assay is designed for quantifying the activation or inhibition of various signaling pathways and provide a downstream (transcription/ translational) read-out that is complementary to upstream receptor-based assays to gain a comprehensive understanding of drug molecule's MOA

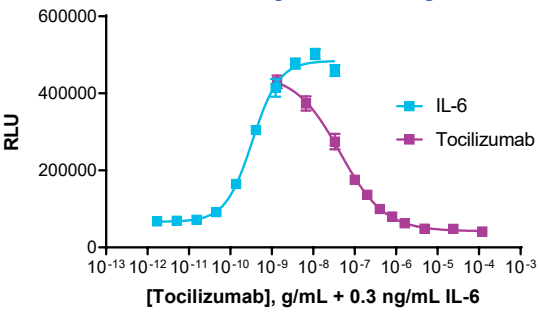


PathHunter® HepG2 STAT3 Pathway Reporter Assay

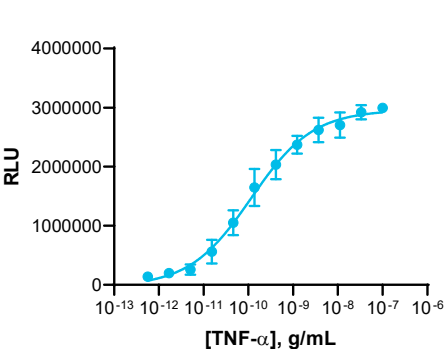
IL-6 Agonist Testing



IL-6R Antagonist Testing

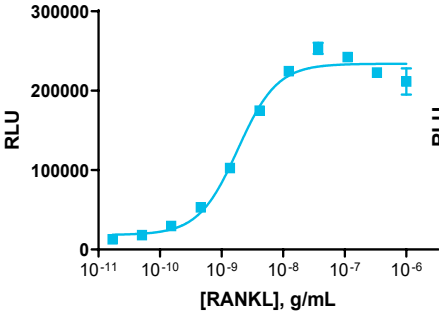


PathHunter HEK293 NF-κB Pathway Reporter Assay

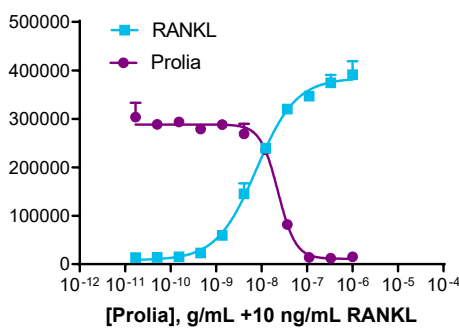


PathHunter U2OS RANK NF-κB Pathway Reporter Assay

RANKL Agonist Testing



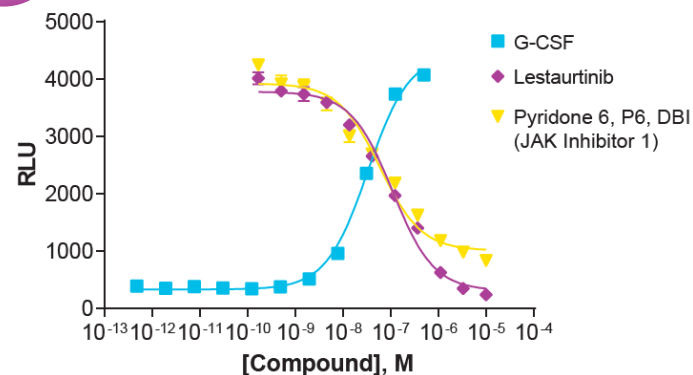
RANKL Antagonist Testing



PathHunter® Cell-Based Functional Assays for Small Molecule JAK Inhibitors

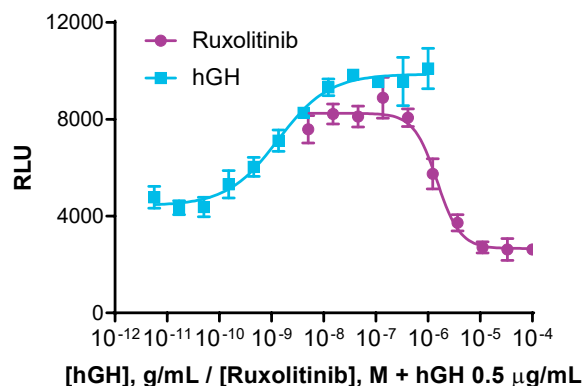
- There are several active, randomized, controlled trials evaluating the therapeutic potential of JAK inhibitors for treatment of COVID-19 (e.g. Ruxolitinib, Baricitinib)
- JAK inhibitors can have a distinct advantage over other immunomodulatory strategies in COVID-19, as they can exert a dual effects: anti-inflammatory (blockade of multiple, pro-inflammatory cytokines at the same time) and anti-viral effects (inhibiting cellular viral endocytosis)

JAK1 PathHunter U2OS CSF3R-JAK1 Functional Assay



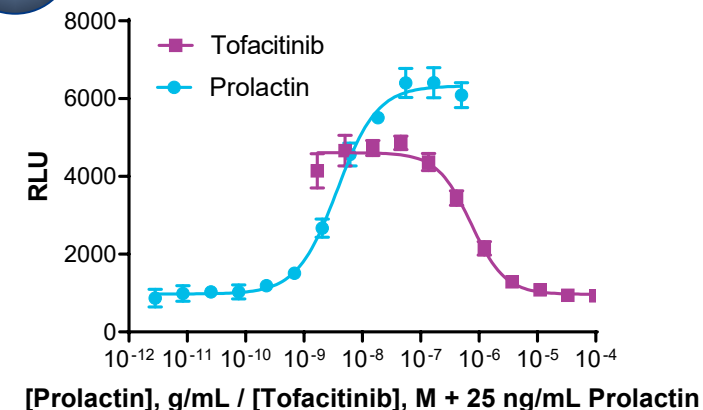
	EC ₅₀ /IC ₅₀	S/B
G-CSF	9 ng/mL	13
Lestaurtinib	0.2 µg/mL	12
Pyridone 6	0.25 µg/mL	4.5

JAK2 PathHunter U2OS TYK2 Activity Assay



	EC ₅₀ /IC ₅₀	S/B
hGH	1.3 ng/mL	2.8
Ruxolitinib	1.4 µg/mL	3.1

JAK3 PathHunter U2OS JAK3 Activity Assay



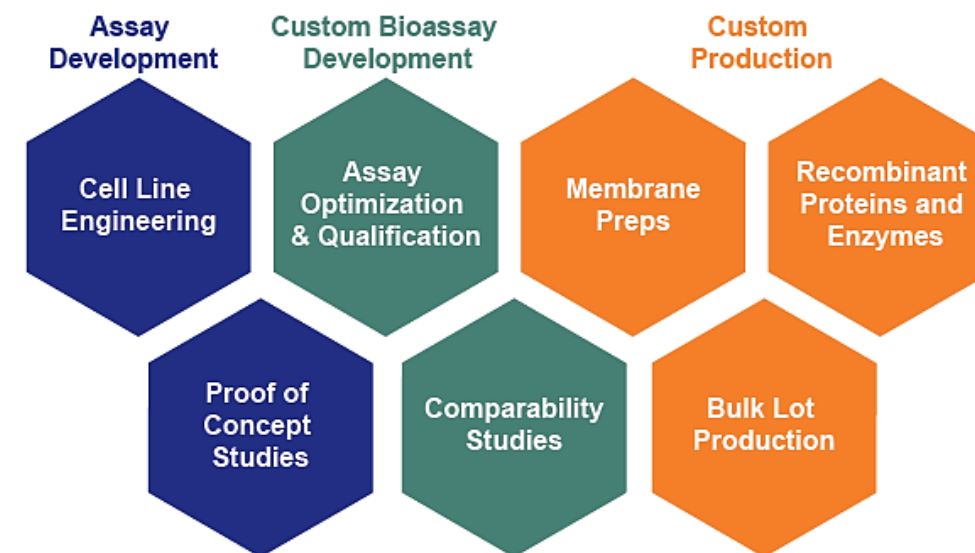
	EC ₅₀ /IC ₅₀	S/B
Prolactin	3.7 ng/mL	7.4
Ruxolitinib	0.7 µg/mL	5.2



Your Target Biology, Our Expertise — Building a Better Assay Together

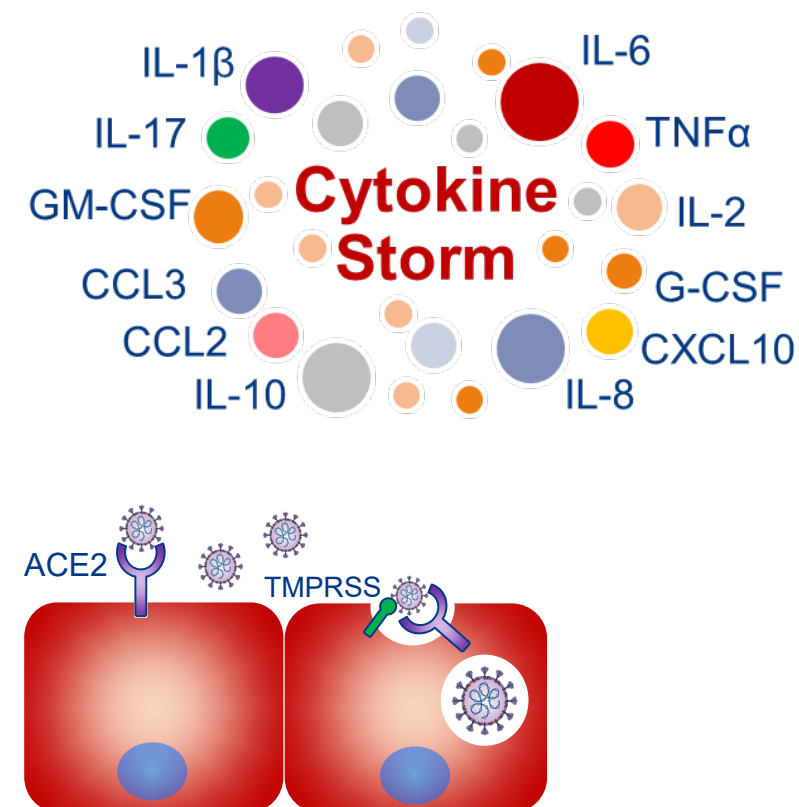
- **Development Expertise** — Decades of cell-based assay development, cell line engineering, and recombinant enzyme development expertise
- **Cell Line Engineering Capability** — Exogenous expression approaches (constitutive vs inducible) or gene editing (e.g. KO/KI with CRISPR/Cas9)
- **Collaborative** — Consultative assay development with regular updates through a dedicated project manager
- **Complete Solution** — Customized assay development with screening and profiling services within the same team

CAD Services Capabilities



Accelerate your drug discovery program with qualified, ready-to-use cell-based interleukin and cytokine assays for Covid-19 research

- **Target-Specific** – MOA-reflective, functional assays for drugs targeting pro-inflammatory cytokines, such as IL-1, IL-6, IL-7, GM-CSF and TNF α , all of which are implicated in COVID-19 pathology
- **Qualified Assays** – Robust, reproducible off-the-shelf assays qualified with innovator drugs
- **Complete Solutions** – Custom Assay Development enabling overexpression of other COVID-19-relevant receptors such as ACE2, TMPRSS2, etc.



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Thank You!



In Stock:

All Products Ready to Ship



Cell Line Rental Program:

3-Months Block



Proof-of-Concept:

Feasibility Study



CRO Certification Program:

Method Transfer Support to CROs



Cell Banking Service for Bioassays:

Ensuring Long-Term Assay Reproducibility for Critical Reagents



Quick Confirmation:

Ready-to-Plate Assay Format

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Products: www.discoverx.com

Services: www.eurofinsdiscoveryservices.com

Stable Cell Lines

Qualified Bioassays

MOA-based Bioassays

Analytical Cell Banks

Custom Assay Development

GPCRs

Checkpoint Receptors

Cytokine Receptors

Kinases

Signaling Pathways

TGF β Superfamily

ADCC Assays

ADCP Assays

CDC Assays

T – Cell Redirection

CD16 Effector Cells