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Accelerating Development of Therapeutic Interventions for COVID-19

Gaurav Agrawal, Ph.D.

Scientific Development Manager, Eurofins DiscoverX

Eurofins DiscoverX is a Global Leader in Supporting Early to Late Stage Drug Discovery

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From Discovery to Development to Clinic to Post-Market

20+ years of enabling drug discovery and development programs

10+
Druggable target classes

1500+
Stable cell line and membrane preps

20+
core patents

Validated
> 30 Billion data points screened in assay services with same assays

2000+
Publications across multiple applications

55+
Qualified and MOA Based Bioassays

ICH Based Bioassay Qualification
Facilitate downstream validation studies

Certified CRO Partners
Scientific training to enable global CROs

Dedicated Scientific Support
Experienced team providing scientific support

20+ Successful Assay Transfers
At clients/affiliated CRO sites

50+ Global Programs
For potency, stability and Nab testing

Global programs using DiscoverX Assays for potency, stability and NAb testing for Drug Release

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SIRPα Bioassay
Multiple Programs in North America for originator biologics

Nab assessment programs at CROs

PD-1 Bioassay
Multiple Programs in North America and APAC

Clinical Development of Biosimilars

VEGF Bioassay
Multiple Programs in North America, EU and APAC

Clinical Development of Biosimilars

Insulin Bioassay
Multiple Programs in and APAC for clinical Development of Biosimilars

CNR1 Bioassay
US based Biopharma for originator biologic

GLP1R Bioassay
Multiple Programs in North America, EU and APAC

Clinical Development of Biosimilars in NA

IGF1R Bioassay
Multiple Programs in North America and APAC for originator biologic

Bradykinin Bioassay
US based CRO for undisclosed client

PTHR1 Bioassay
Multiple Programs in North America, EU and APAC

Clinical Development of Biosimilars

FGFR Bioassay
Nab assessment program for US based Biopharma

ErbB2/ErbB3 Bioassay
Multiple Programs in North America, EU and APAC

Clinical Development of Biosimilars

Melanocortin Bioassay
European Biopharma for an originator biologic

IL-1 Bioassay
Multiple Programs in NA for originator biologic

IL-2 Bioassay
Multiple Programs in North America and EU for originator biologic

IL-7 Bioassay
European Biopharma for originator biologic

IL-10 Bioassay
Multiple Programs in North America for originator biologic

IL-31 Bioassay
US based pharma for Nab assessment and potency testing programs for originator biologic

RANK Assay
Multiple Programs in and APAC for clinical Development of Biosimilars

GM-CSF Bioassay
Multiple Programs in EU and NA for originator biologic

Largest Menu of Cell-Based Assays for Discovery, Potency, & NAb Assay Development

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>800 cell lines to support bioassay development for major drug target classes

289
Opioid
Glucagon
GABA
Neuropeptide
Chemokine
GPCRs

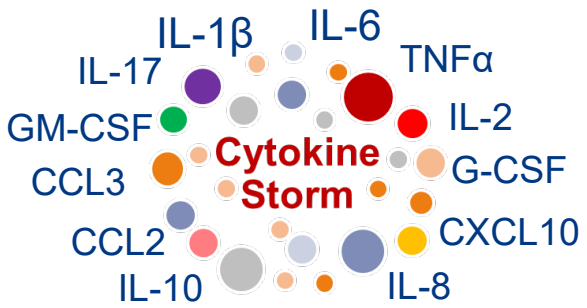
55
IGF1R
Tie2
ErbB1
ErbB2/B3
VEGFR
RTKs

30+
NRF2
TGFβ
IκB
SREBP
PI3
Pathway

8
NFkB
STAT3
NFAT
Signaling Reporter

33
SIRPα
PD-1
CTLA-4
BTLA
OX40
Checkpoints

54
ADCC
ADCP
T-cell Redirection
CDC
Effector Cells
Cytotoxicity

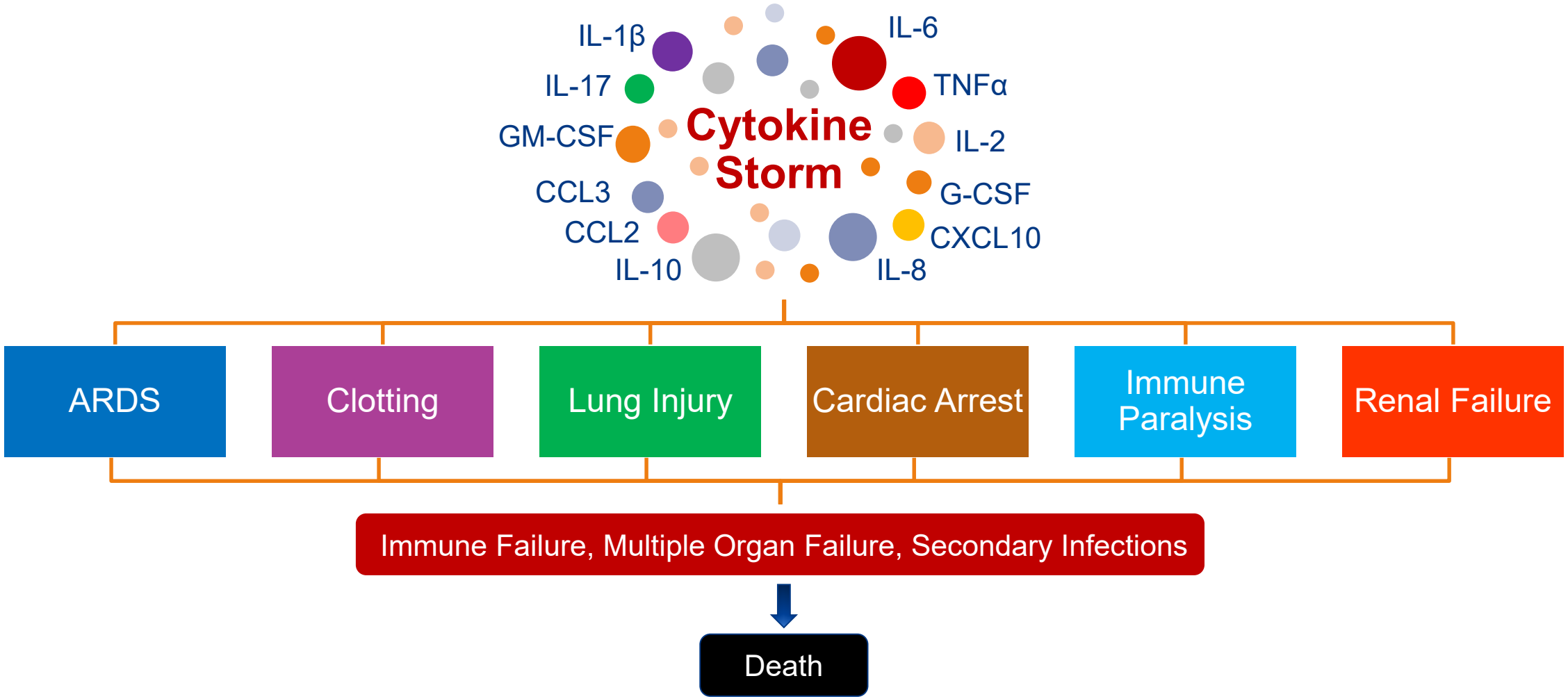


Cytokines & Interleukins

53
Keytruda®
Humira®
Victoza®
Avastin®
Vectibix®
Bioassays

Qualified with innovator drug

Cytokine Storm in COVID-19 Pathology



Withstanding the Cytokine Storm in COVID-19: Therapeutic Approach

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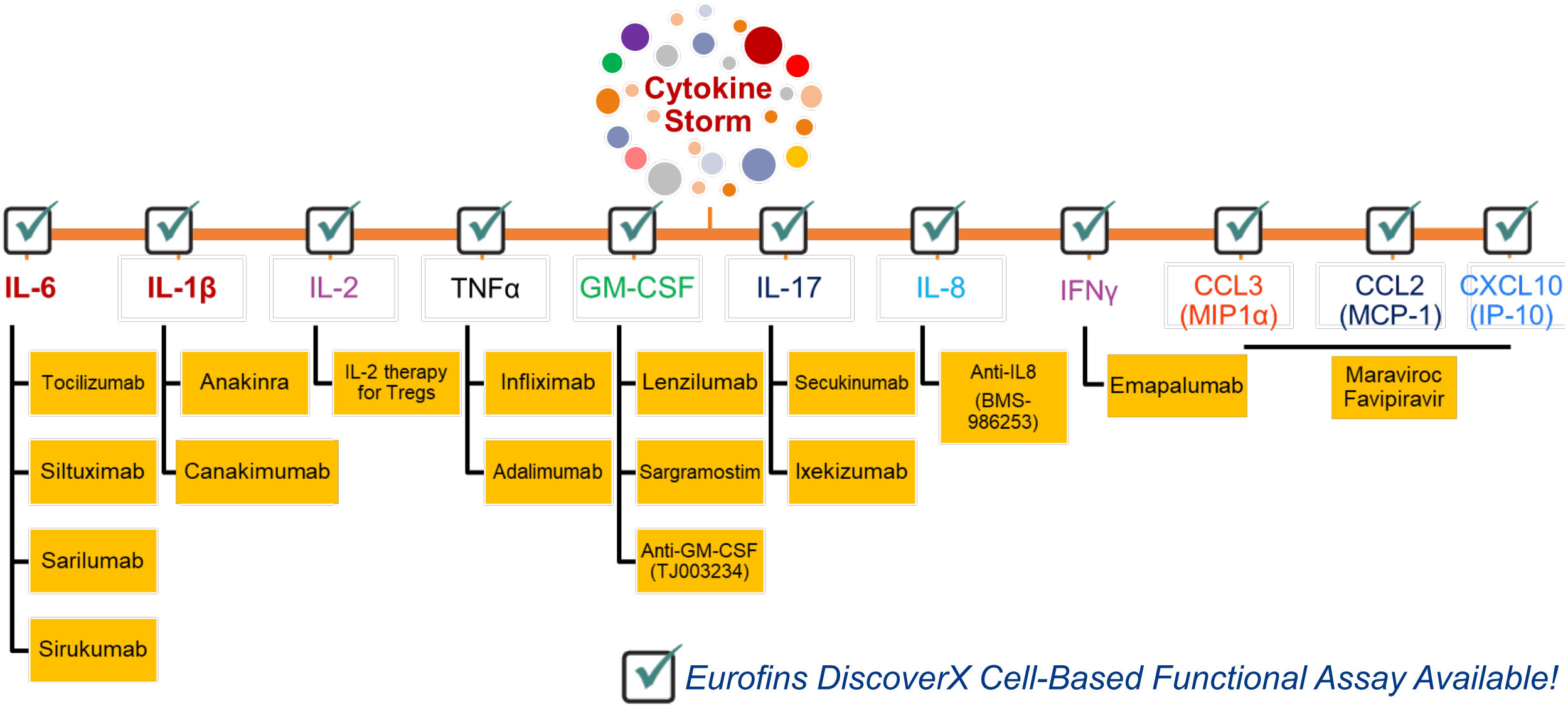

Eurofins DiscoverX Cell-Based Functional Assay Available!

Accelerating Implementation Phase for QC Lot Release with Qualified Bioassays

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Typical Bioassay Development Timeline

Time to Market >12 months

Characterize Cell Line	Assay Optimization	Testing Innovator Drug	Assay Qualification	Lot/s Manufacture	Method Transfer (Client/CRO)	Assay Validation	QC Lot Release
• Choose assay format that best reflects MOA of drug	• Optimize assay parameters (thaw-and-use cells)	• Refine conditions with innovator drug	• Establish robustness, precision, accuracy, and linearity of assay	• Generate lots for bioassay validation	• Method review • On-site transfer runs	• ICH Validation (GMP)	

Eurofins DiscoverX Bioassay Development Timeline

Time to market <3 months

Characterize Cell Line	Assay Optimization	Testing Innovator Drug	Assay Qualification	Lot/s Manufacture	Method Transfer (Client/CRO)	Assay Validation	QC Lot Release
☑ **DONE** Cell Line established	☑ **DONE** Bioassay developed	☑ **DONE** Bioassay optimized with innovator drug	☑ **DONE** Qualification complete	☑ **DONE** Lots available for immediate use	• Method review • On-site transfer runs	• ICH Validation (GMP)	

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

Analytical Assays for Therapeutics Targeting Key Proinflammatory Cytokines

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1. **Qualified Bioassays:**
   - Case Study with PathHunter® Tocilizumab Bioassay - Qualified with ACTEMRA® tocilizumab for therapeutics targeting IL-6 pathway
   - IL-1β - PathHunter® Anakinra Bioassay - Qualified with Kineret® (anakinra)
   - GM-CSF - PathHunter® Sargramostim Bioassay – Qualified with Leukine® sargramostim A Recombinant GM-CSF–Yeast-Expressed
   - TNFα - PathHunter® Adalimumab Bioassay - Qualified with HUMIRA adalimumab
2. **Signaling Reporter Assays**
   - IL-6
   - TNFα
   - RANK (TNFSF11)
3. **Functional Cell-Based Assays for Small Molecule Inhibitors**
   - JAK1, JAK2 and JAK3

Technology Overview

Enzyme Fragment Complementation

Enzyme Fragment Complementation (EFC) Platform

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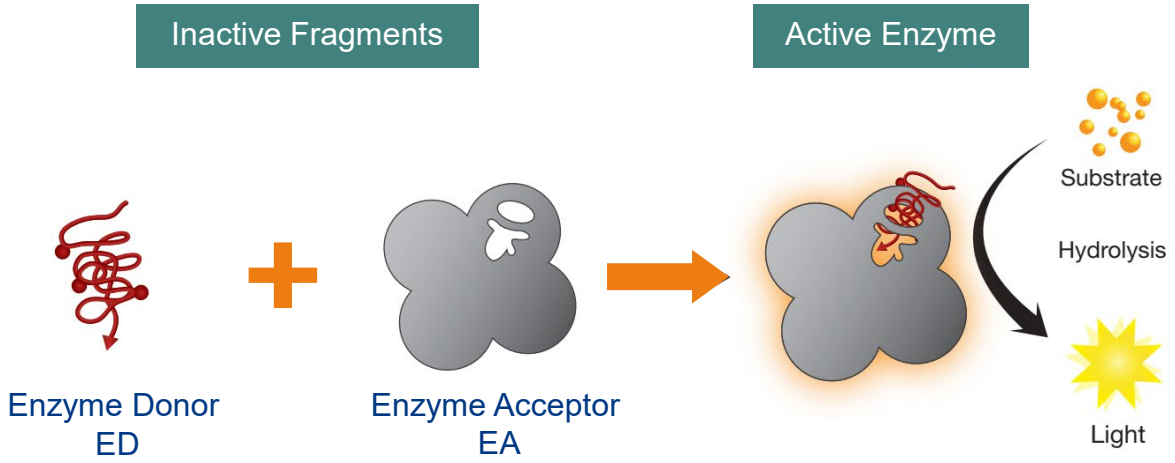
Enabling Technologies with a Flexible Platform based on a Split β-Galactosidase Enzyme



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

MOA-Based Assays Design using Enzyme Fragment Complementation Technology

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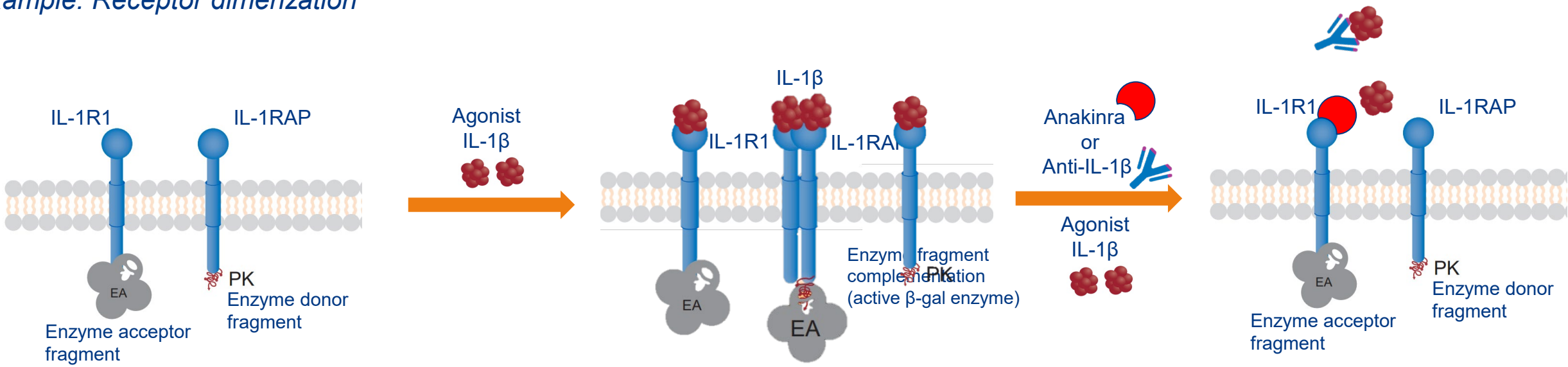
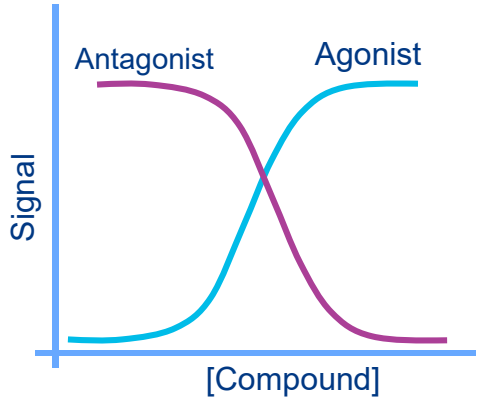
Example: Receptor dimerization





Quantitative response

Enzyme Fragment Complementation: a Versatile and Robust Platform for Cell-Based Assays

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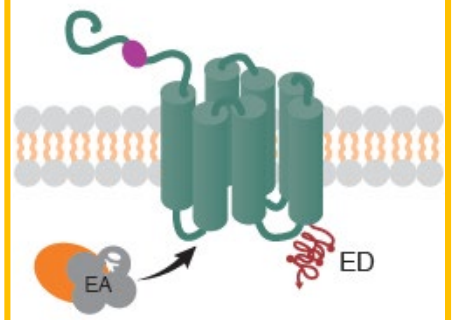
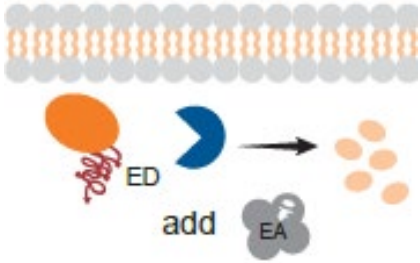
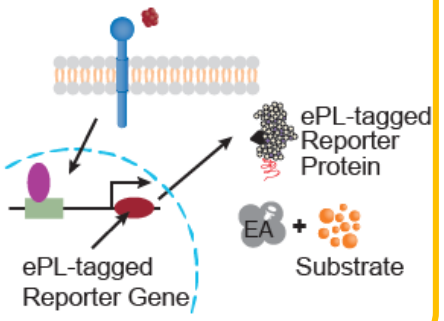
Protein-Protein Interaction



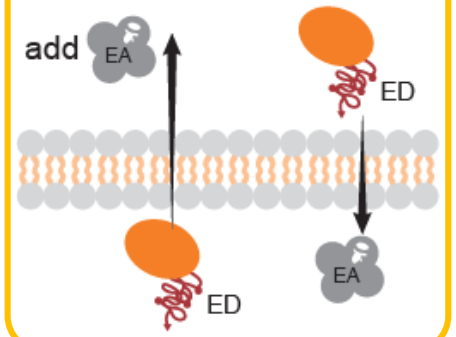
Quantitation and Degradation



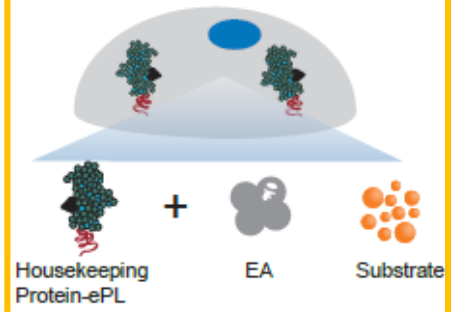
Downstream Response Evaluation



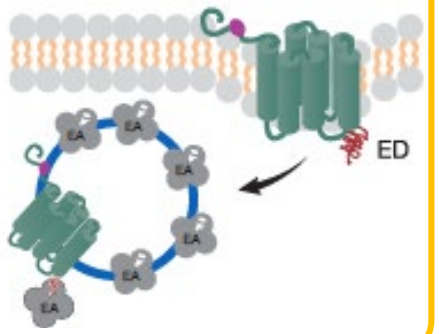
Secretion and Translocation



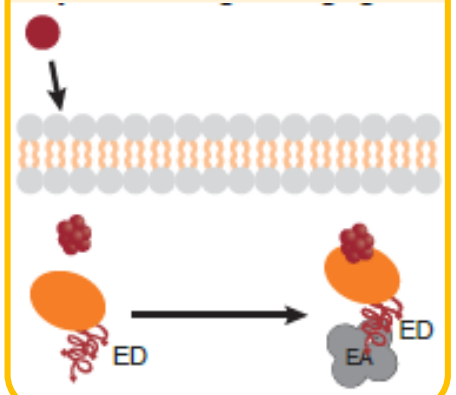
Quantify Cytotoxicity



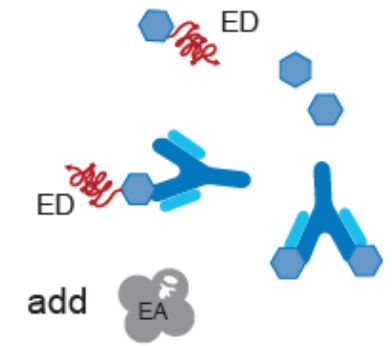
Internalization and Trafficking



Compound Target Engagement



Analyte Detection



Flexibility of Formats - Phase-Appropriate Solutions for Every Development Program

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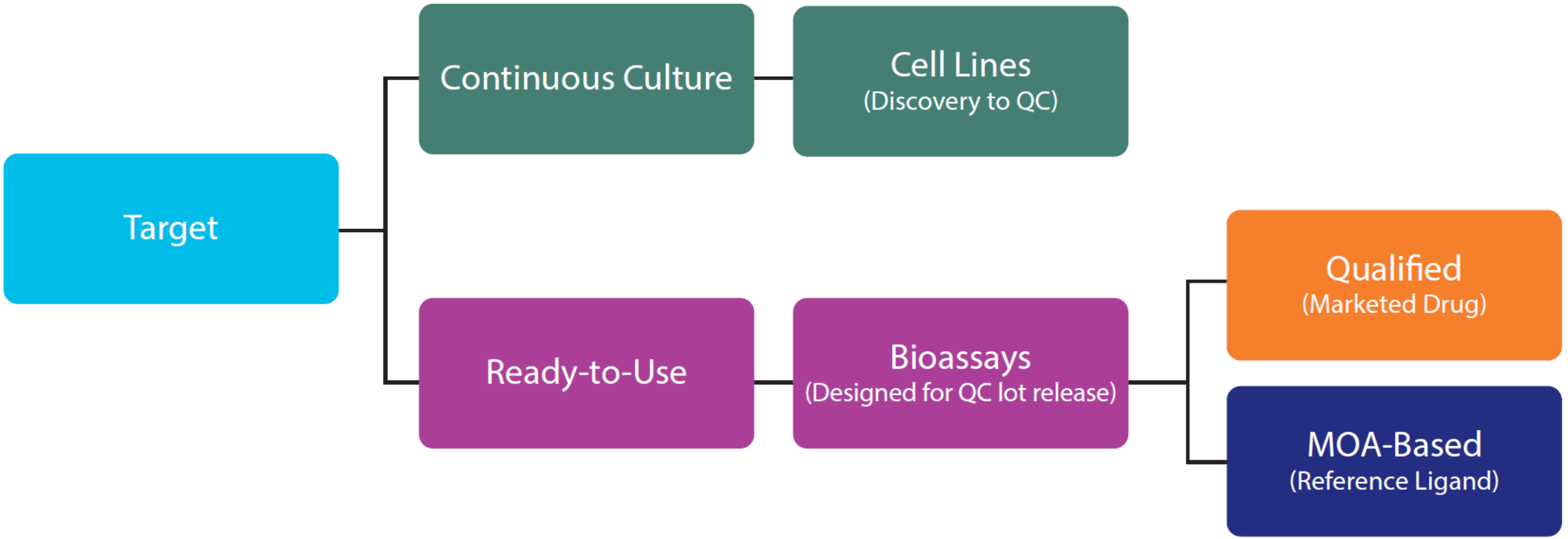
From Continuous Culture to Qualified Bioassays



Eurofins DiscoverX Qualified Bioassays are designed for QC Lot Release Programs

EFC Cell-Based Assays Protocol

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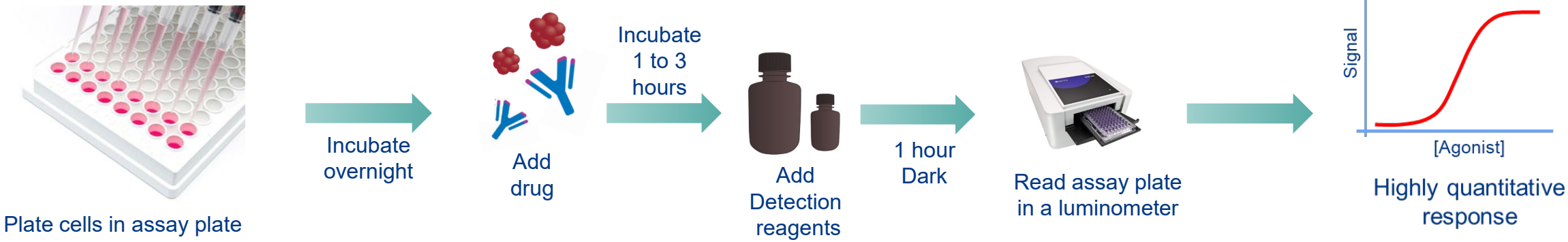
Typical assay protocol for stable cell lines and assay ready kits



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®

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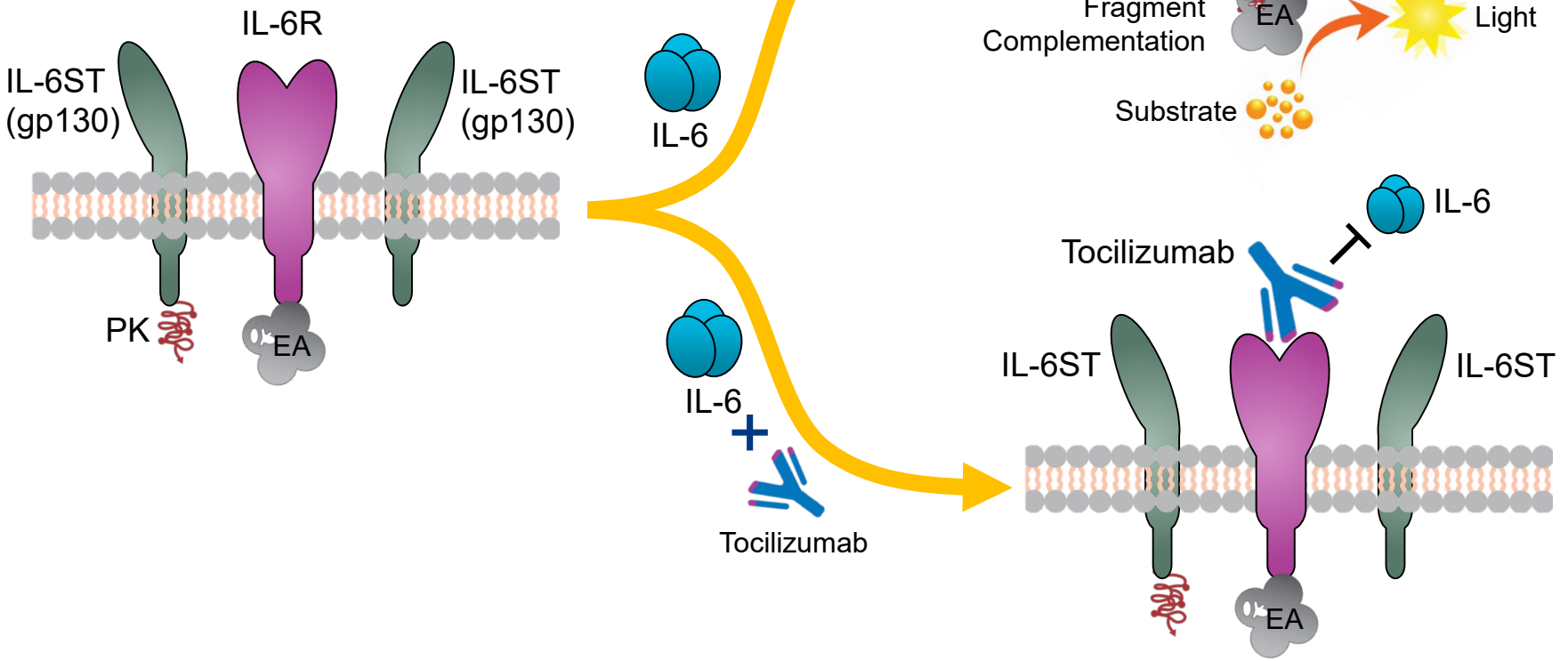
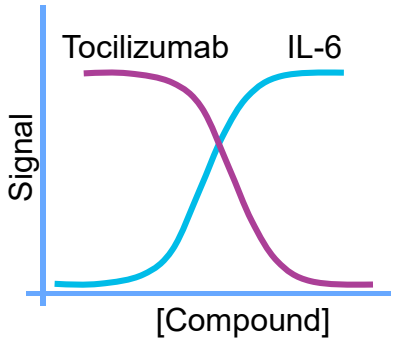
MOA-reflective Assay Design: Receptor Dimerization

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)





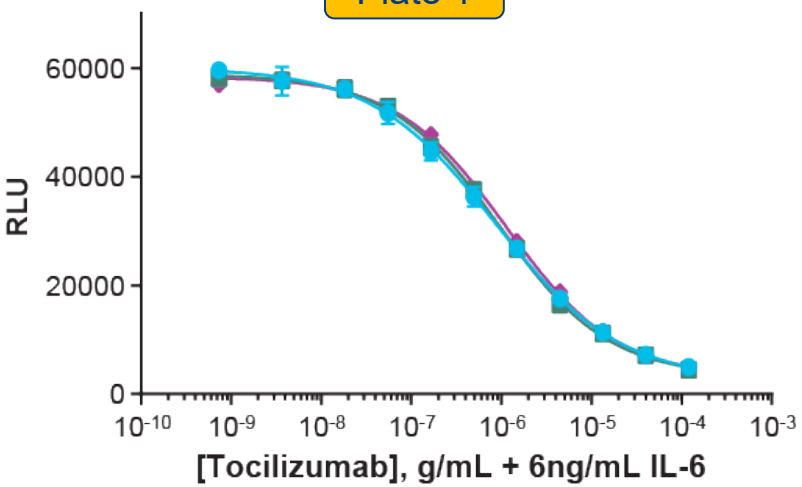
Assay Parameters Assessed for Qualification

- 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% preformed by two operators
- Parallel line analysis using PLA3.0 or SoftMax Pro 7.1

Assessing Intra-Day Plate-to-Plate Variability

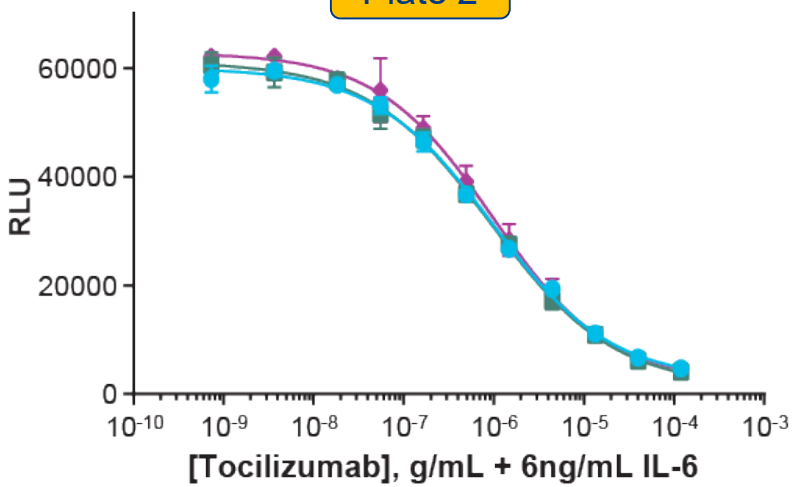
Experiments depicting 3 assay plates with full-plate DRC - Same Day – One operator

Plate 1



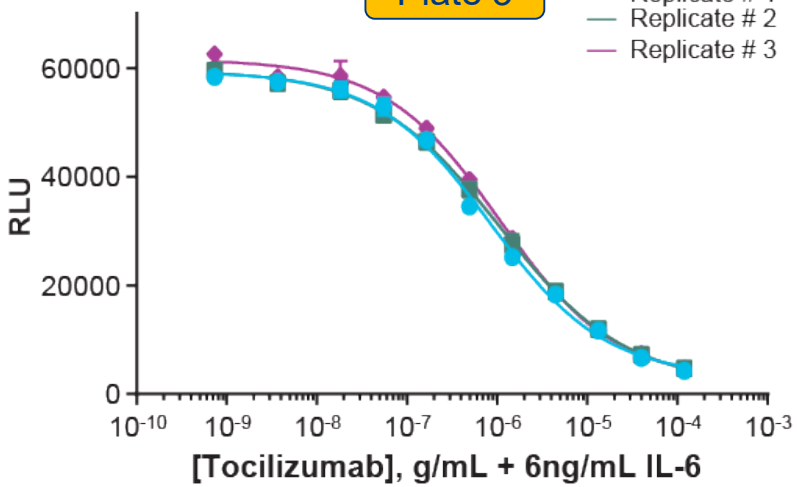
Parameter	R1	R2	R3
S/B	12.1	13.7	12.6
IC_{50} (ng/mL)	868	935	1,131

Plate 2



Parameter	R1	R2	R3
S/B	13.2	15.2	14.1
IC_{50} (ng/mL)	966	943	972

Plate 3



Parameter	R1	R2	R3
S/B	14.5	12.8	13.8
IC_{50} (ng/mL)	850	1077	1063

Assay Robustness Analysis

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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): 9.7%

Intermediate Precision (Inter-Day): 24.6%

Parallelism and Relative Potency Estimation

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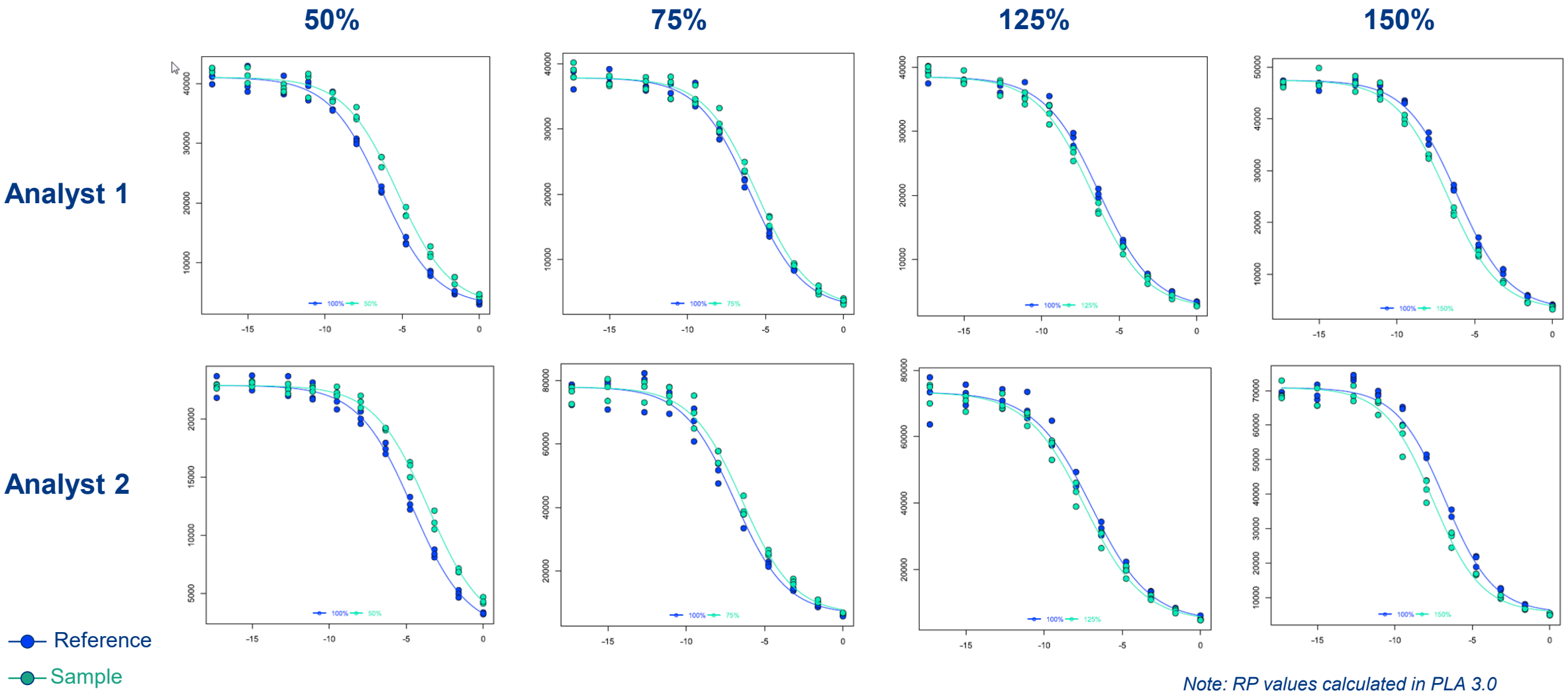
Relative Potency: Parallelism and Potency Estimation (PLA)



Note: RP values calculated in PLA 3.0

Tocilizumab Bioassay: Qualification with Actemra®

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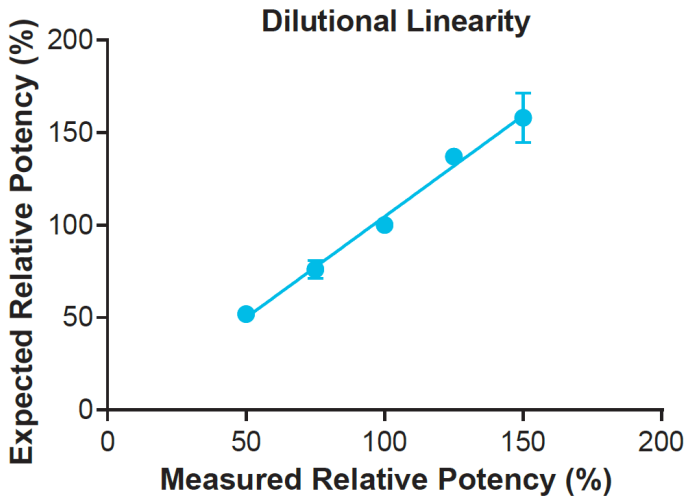
Summary: Accuracy, Precision and Dilutional Linearity (Single Analyst)

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%

Tocilizumab Bioassay is highly accurate, precise and linear!

Qualified Bioassays for Precise Potency Determination for Cytokines and Interleukins

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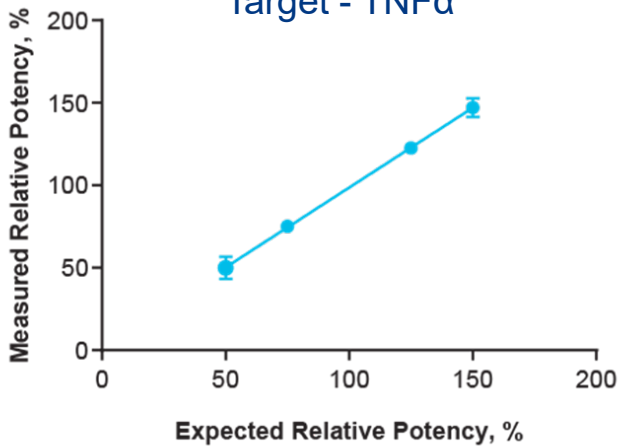
ICH Based Assay Qualification

- Accuracy
- Precision
- Range
- Dilutional Linearity
- Parallelism
- Specificity

HUMIRA adalimumab

Adalimumab Bioassay Qualification

Target - TNFα



Dilutional Linearity: 99.99%

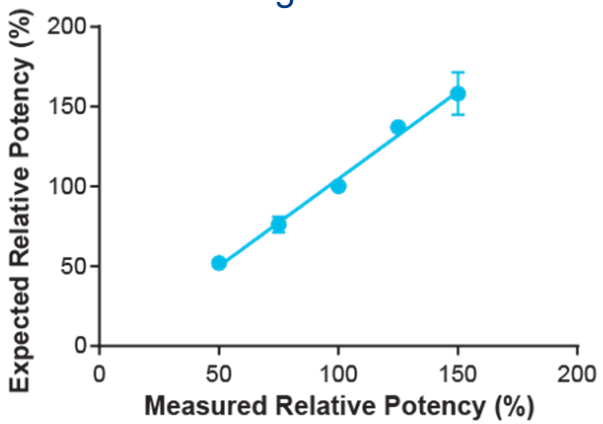
Accuracy: 105.1%

Precision: 5.65%

Kineret® (anakinra)

Anakinra Bioassay Qualification

Target – IL-1



Dilutional Linearity: 96.7%

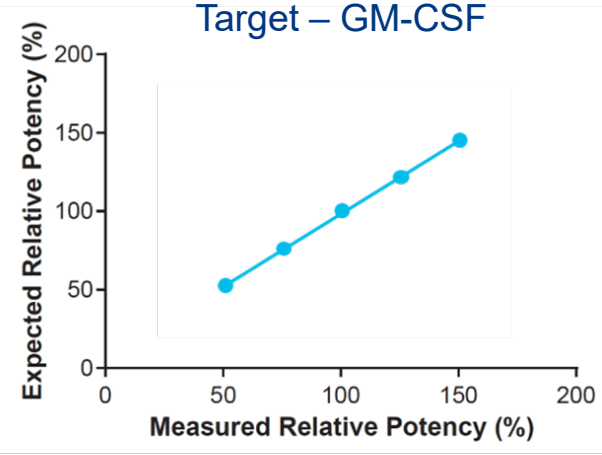
Accuracy: 99.4%

Precision: 11.4%

Leukine® sargramostim
A Recombinant GM-CSF–Yeast-Expressed

Sargramostim Bioassay Qualification

Target – GM-CSF



Dilutional Linearity: 99.56%

Accuracy: 99.1%

Precision: 3%

Eurofins DiscoverX Bioassays are qualified with excellent linear potency as demonstrated with clinically relevant drugs

Signaling Pathway Reporter Assays for Cytokines and Interleukins

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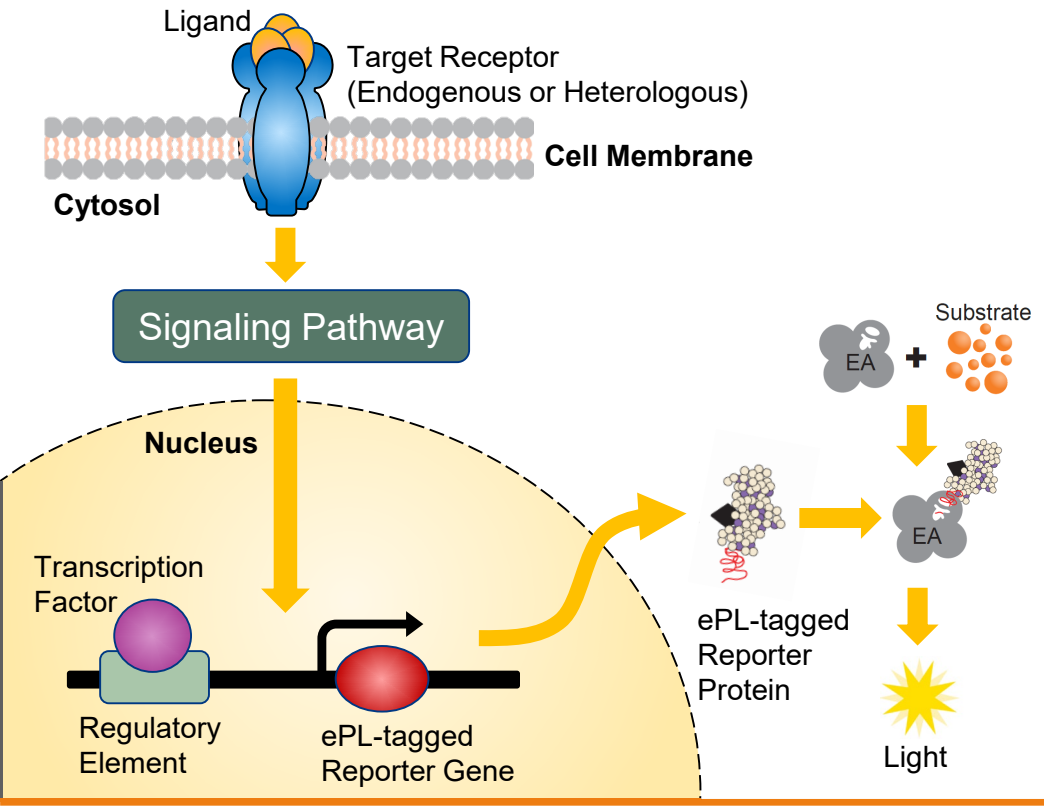
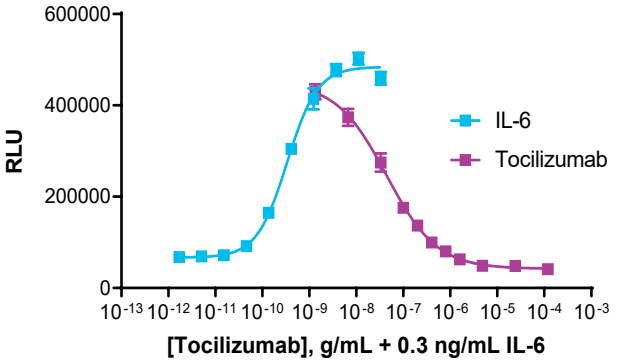
Assay Design : Signaling Reporter

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

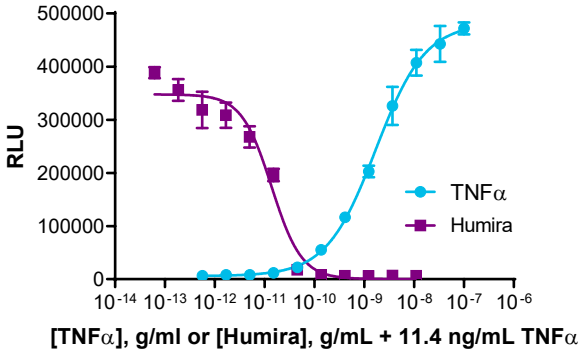


PathHunter® HepG2 STAT3 Pathway Reporter Assay



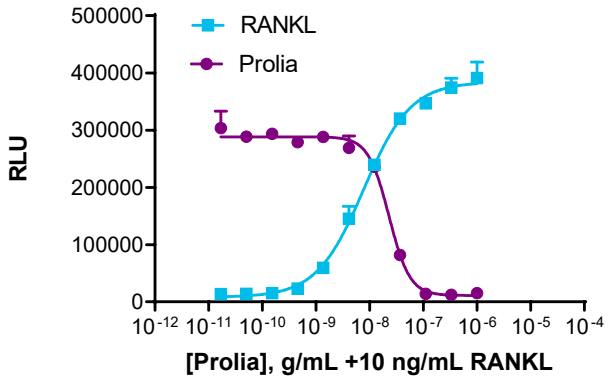
	EC_{50}/IC_{50}	S/B
IL-6	0.33 ng/mL	6.2
Tocilizumab	44 ng/mL	11.6

PathHunter U2OS NF-κB Pathway Reporter Assay



	EC_{50}/IC_{50}	S/B
TNFα	1.7 ng/mL	72.3
Humira	13 pg/mL	64

PathHunter U2OS RANK NF-κB Pathway Reporter Assay

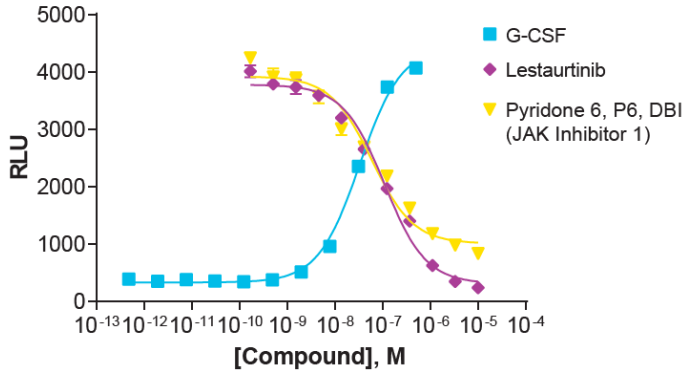


	EC_{50}/IC_{50}	S/B
RANKL	7.7 ng/mL	27
Prolia	23 ng/mL	21

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

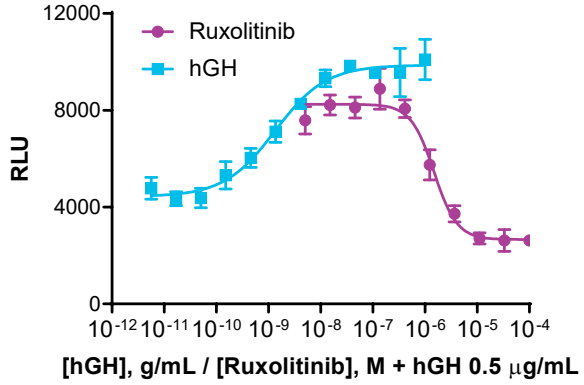
- There are several active, randomized, controlled trials evaluating the therapeutic potential of JAK inhibitors for treatment of COVID-19 (e.g. Ruxolitinib, Baricitinib)
- JAK inhibitors can have a distinct advantage over other immunomodulatory strategies in COVID-19, as they can exert a dual effects: 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



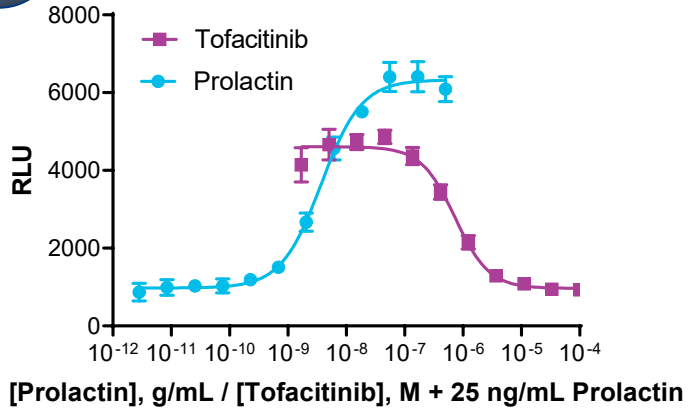
	EC_{50}/IC_{50}	S/B
G-CSF	9 ng/mL	13
Lestaurtinib	0.2 µg/mL	12
Pyridone 6	0.25 µg/mL	4.5

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

Custom Assay Services

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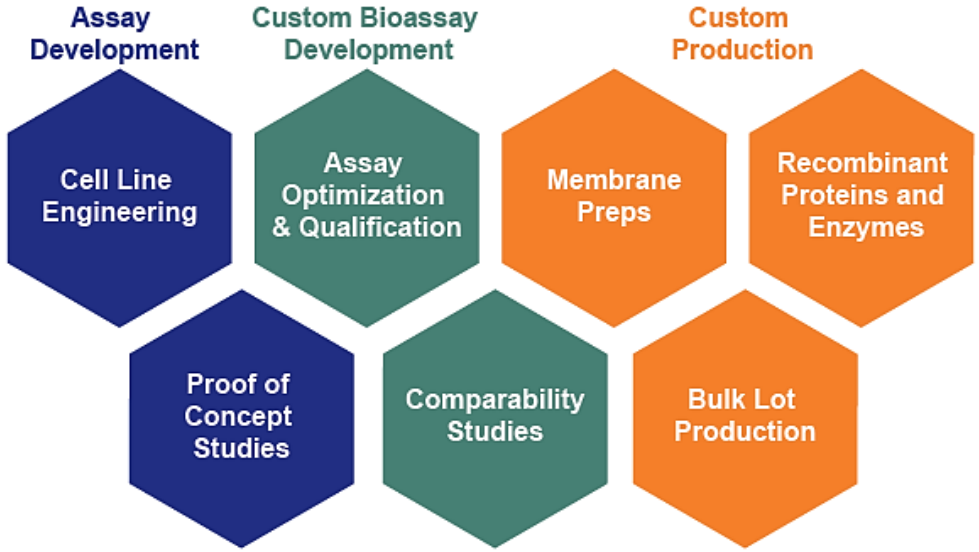
Your Target Biology, Our Expertise — Building a Better Assay Together

- **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



Conclusions

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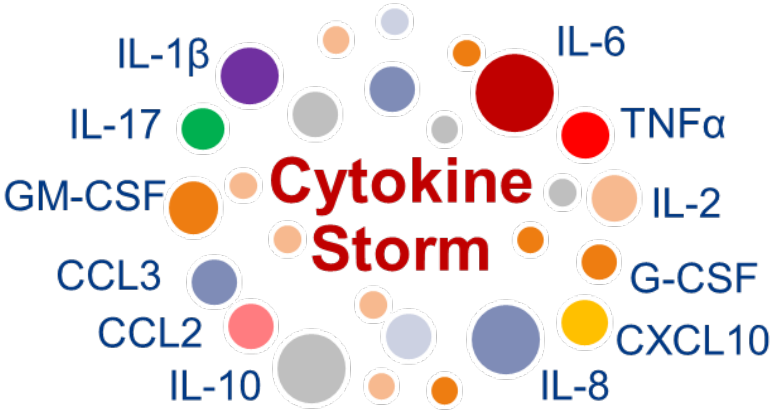
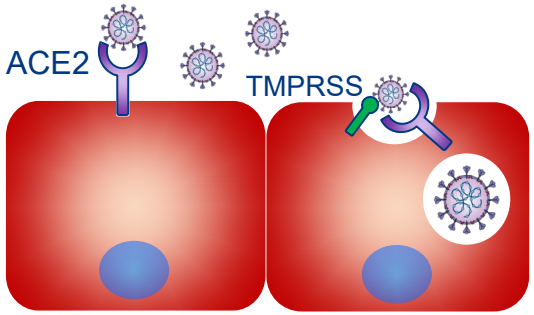
Accelerate your drug discovery program with qualified, ready-to-use cell-based interleukin and cytokine assays for Covid-19 research

- **Target-Specific** – MOA-reflective, functional assays for drugs targeting pro-inflammatory cytokines, such as IL-1, IL-6, IL-7, GM-CSF and TNFα, 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.





Visit **discoverx.com/covid-19** to learn more

Accelerating Implementation Phase for QC Lot Release with Qualified Bioassays

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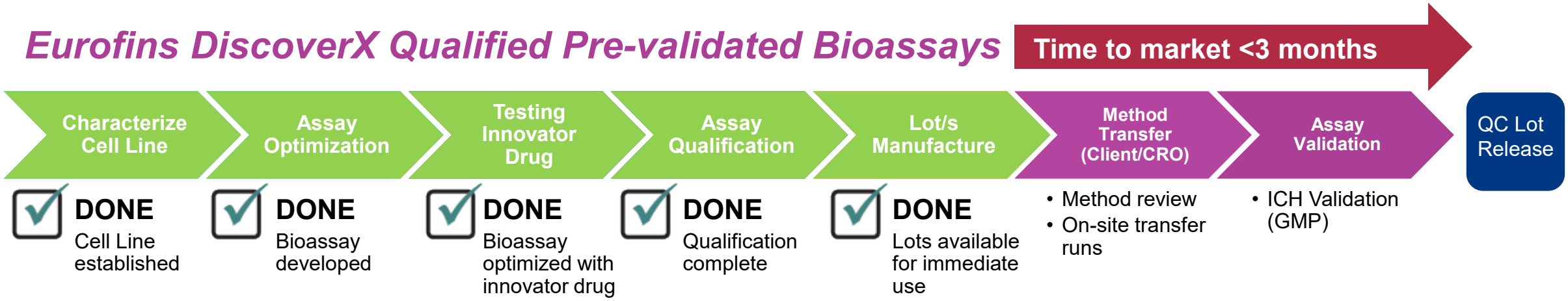
Typical Bioassay Development Timeline



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

Eurofins DiscoverX is a Global Leader in Supporting Early to Late Stage Drug Discovery

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From Discovery to Development to Clinic to Post-Market

20+ years of enabling drug discovery and development programs

10+
Druggable target classes

1500+
Stable cell line and membrane preps

20+
core patents

Validated
> 30 Billion data points screened in assay services with same assays

2000+
Publications across multiple applications

55+
Qualified and MOA Based Bioassays

ICH Based Bioassay Qualification
Facilitate downstream validation studies

Certified CRO Partners
Scientific training to enable global CROs

Dedicated Scientific Support
Experienced team providing scientific support

20+ Successful Assay Transfers
At clients/affiliated CRO sites

50+ Global Programs
For potency, stability and Nab testing