

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

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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.
- COVID-19 patients in severe cases shows aggressive inflammatory response against the infected tissue and can causes long term organ damage. Therefore, several ongoing clinical programs are evaluating therapeutics targeting the inflammatory signals as a treatment strategy for COVID-19.
- 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 and may cause Acute respiratory distress syndrome (ARDS). This can lead directly to respiratory failure.
- Proinflammatory cytokine levels are significantly elevated in patients with ARDS, and the degree of increase is positively correlated with mortality rate.



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Proinflammatory Cytokine Pathogenesis of COVID-19



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Withstanding the Cytokine Storm in COVID-19: Therapeutic Approach





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Qualified Bioassays:

- Case Study with PathHunter[®] Tocilizumab Bioassay Qualified with ACTEMRA[®] for therapeutics targeting IL-6 pathway
- IL-1 β PathHunter[®] Anakinra Bioassay Qualified with $\bigotimes_{(a n a k i n r a)}^{\text{Kineret}^{\circ}}$
- GM-CSF PathHunter[®] Sargramostim Bioassay Qualified with Leukine[®] sargramostim
- TNFα PathHunter[®] Adalimumab Bioassay Qualified with HUMIRA

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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

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

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

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From Continuous Culture to Qualified Bioassays



Eurofins DiscoverX Qualified Bioassays are designed for QC Lot Release Programs

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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



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



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

PathHunter[®] Tocilizumab Bioassay - Qualified with Actemra[®]



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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: EC₈₀ and IC₈₀ (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

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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

Intermediate Precision (Inter-Plate): 9.7%

Intermediate Precision (Inter-Day): 24.6%



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RL



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

Parallelism and Relative Potency Estimation

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Cerep | DiscoverX | Panlabs | EID | Villapharma

Tocilizumab Bioassay: Qualification with Actemra®

Construction Const

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			



Tocilizumab Bioassay is highly accurate, precise and linear!

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

Construction Const



Kineret[®] is a registered trademark of Amgen, Inc

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40000-

30000-

20000-

RLU

Bioassay measures a robust response to IL1β and Anakinra.

Functional Data

Anakinra Bioassay is highly accurate, precise and linear

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

IL-1β

Anakinra

Leukine® is a registered trademark licensed to Genzyme Corporation

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Case Study 4: TNFα

RLU

	3/D	EC ₅₀ / IC ₅₀
TNFα	10.3	53.9 pM

PathHunter[®] Adalimumab Bioassay - Qualified with Humira[®]

Bioassay measures a robust response to TNFα and Humira

Adalimumab Bioassay is highly accurate, precise and linear

Precision: 5.65%

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Humira[®] is a registered trademark AbbVie, Inc

Accelerating Implementation Phase for QC Lot Release with Qualified Bioassays

Typical Bioassay Development Timeline

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

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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

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 <u>Custom Assay Development</u> enabling overexpression of other COVID-19-relevant receptors such as ACE2, TMPRSS2, etc.

Visit discoverx.com/interleukins to learn more

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Thank You!

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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

Stable Cell Lines Qualified Bioassays

MOA-based Bioassays

Analytical Cell Banks

Custom Assay Development

GPCRs

Checkpoint Receptors

Cytokine Receptors

Kinases

Signaling Pathways

TGFβ Superfamily

ADCC Assays

ADCP Assays

CDC Assays

T – Cell Redirection

CD16 Effector Cells

Learn more about our available cell-based assays Products: <u>www.discoverx.com</u> Services: <u>www.eurofinsdiscoveryservices.com</u>