InCELL Pulse: Cellular Target Engagement Assay Platform for Drug Discovery

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Abstract

It is well established that ligand binding can protect proteins from thermal denaturation in a cellular milieu; however, the broad application of this cellular target engagement concept to drug discovery has been hindered by a lack of simple platforms with sensitive quantitative readouts. Cell-based thermal stabilization assays are valuable methods for particular applications, but can require target-specific antibodies for immuno-assay readouts, can be low-throughput for Western blot formats, and can be biased against multi-domain proteins. Here we describe InCELL Pulse™, which uses Eurofins DiscoverX Enzyme Fragment Complementation (EFC) technology to overcome these limitations of current thermal stabilization assays. We have successfully applied InCELL Pulse to rapidly measure quantitative cellular target engagement potency values for inhibitors of diverse protein classes, including kinases, methyltransferases, and hydrolases. We further demonstrate broad utility for InCELL Pulse across the human kinome, where we have validated assays for kinases from seven of the eight phylogenetic groups.

InCELL Pulse Cellular Target Engagement Assays

Quantitative thermal denaturation-based cellular ligand binding assays

- Measure cellular inhibitor target-engagement potencies
- Simple, generic cellular alternative to current thermal stabilization assays
- Engineered system not biased against multi-domain proteins
- Rapid one-step readout: No wash, centrifugation, or immunoassay steps
- No custom reagents required (antibodies, chemical tracers)

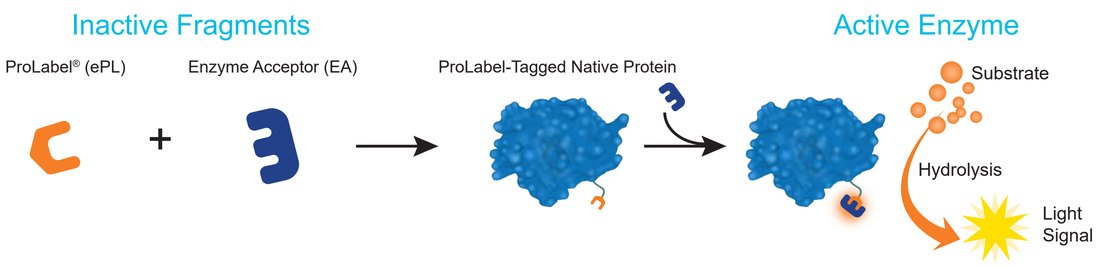
Applications

- Potency rank ordering during lead optimization and HTS hit validation
- Cellular target engagement potency...do compounds enter cell and engage target?
- Ideal for targets difficult to test using functional assays
- De-risk off-targets identified in biochemical screens
- High throughput cellular screening

Validated across diverse target classes

- Hydrolases, methyltransferases, and diverse kinases
- Accurate potency rank order for known inhibitors

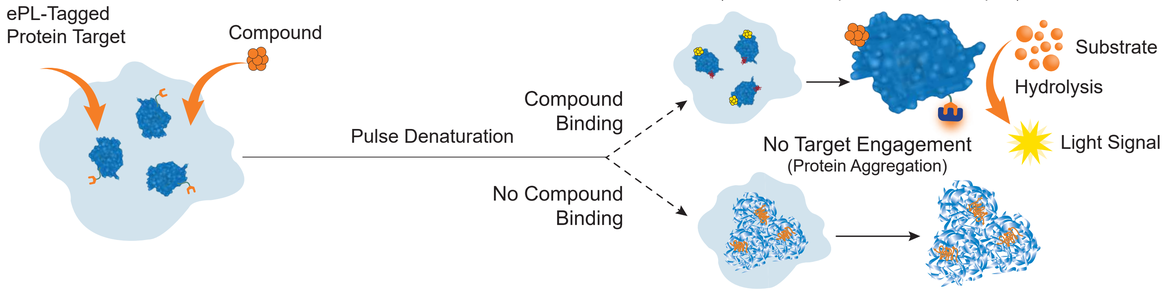
Split β-Galactosidase Enzyme System Supports InCELL Pulse Technology



ProLabel (ePL) is an EFC enzyme donor, dual-purpose peptide tag ideally suited for InCELL Pulse

- ePL tag (~40 aa) allows for sensitive detection of properly folded "native" target protein
- ePL tag senses folded state of protein: Denatured/aggregated proteins poorly complement EA resulting in reduced luminescence

How InCELL Pulse Works



Compound binding protects protein from thermal denaturation and increases EFC in a dose-dependent manner for the measurement of inhibitor EC_{50} values

ePL-tagged target protein expressed in transiently transfected cells

- Live cells treated directly with compound for cellular target engagement studies

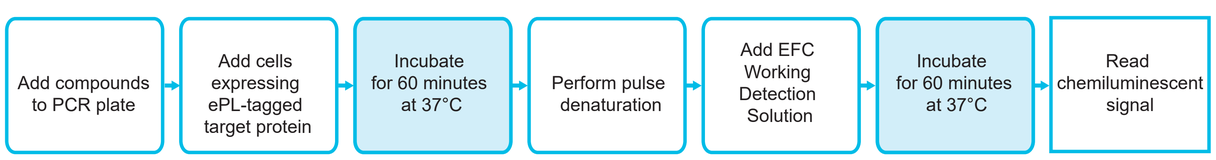
Compound-treated cells subjected to pulse denaturation in thermocycler

- Gentle pulse denaturation protocol does not compromise cell viability or membrane integrity

EFC is performed in conjunction with cell lysis post-pulse denaturation

- Gentle lysis conditions do not refold denatured/aggregated proteins

InCELL Pulse: Rapid and Simple Protocol



InCELL Pulse Overcomes Limitations of Other Cellular Target Engagement Approaches

Simplified assay process with no custom reagent requirements

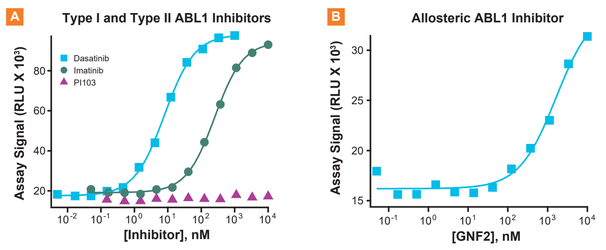
- No centrifugation or immunoassay steps required
- Generic: No custom chemical tracers, antibodies or affinity beads required
- No fusions to functional reporter proteins required

Quantitative power and simplicity of readout

- Superior to immunoassay
- Sensitive, precise, simple, and quantitative with broad dynamic range

InCELL Pulse Assay Validation for Diverse Targets

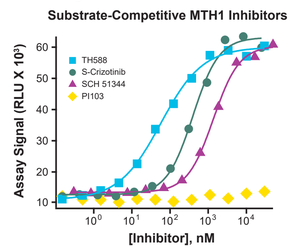
ABL1 Tyrosine Kinase



Inhibitor	Inhibitor Type	EC_{50} (nM)	Assay Window	Hill Slope
Dastatinib	I	8.0	5.4	1.1
Imatinib	II	290	4.8	1.0
GNF2	Allosteric	2,300	2.3	1.0
PI103	Lipid Kinase Inhibitor	>10,000	N/A	N/A

ABL1 tyrosine kinase InCELL Pulse assay data: ABL1 cellular target-engagement dose-response curves for Type I, Type II, and allosteric inhibitors. A. The Type I and Type II inhibitors dasatinib and imatinib, respectively, show the correct rank-order potencies. The lipid kinase inhibitor PI103 was included as a negative control. B. The allosteric inhibitor GNF2, which targets the myristate binding site in the C-terminal kinase lobe, is detected using the ABL1 InCELL Pulse assay.

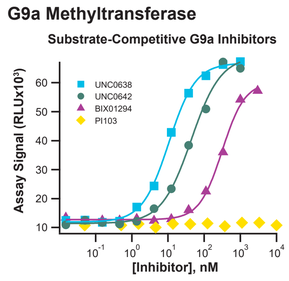
MTH1 Hydrolase



Inhibitor	EC_{50} (nM)	Assay Window	Hill Slope
TH588	44	5.4	0.9
S-Crizotinib	410	5.1	1.4
SCH51344	1,600	4.6	1.1
PI103	>10,000	N/A	N/A

MTH1 hydrolase InCELL Pulse assay data: MTH1 cellular target-engagement dose-response curves for the diverse inhibitors TH588, S-crizotinib, and SCH 51344 show the correct rank order potencies. The lipid kinase inhibitor PI103 was included as a negative control.

G9a Methyltransferase



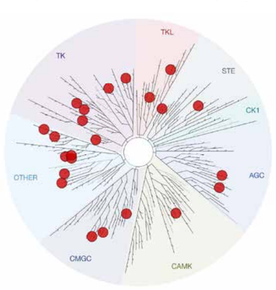
Inhibitor	EC_{50} (nM)	Assay Window	Hill Slope
UNC0638	10	5.3	1.2
UNC0642	36	5.6	1.2
BIX01294	260	4.6	1.4
PI103	>10,000	N/A	N/A

G9a cellular target-engagement dose-response curves for the substrate-competitive inhibitors BIX01294, UNC0642, and UNC0638 show the correct rank-order potencies, with UNC0638 and UNC0642 significantly more potent than BIX01294. The lipid kinase inhibitor PI103 was included as a negative control.

Validated InCELL Pulse Assays for Diverse Kinases

Kinase assay representation on phylogenetic tree

Kinase Target		Class
AAK1	FAK	Tyr Kinase
ABL1	IGF1R	
ABL1(T315I)	JAK2(JH1)	
ACVR1	KIT	
BTK		
AKT1	HASPIN	Ser/Thr Kinase
AURKA	MEK1	
BRAF	p38-α	
BUB1	PAK4	
CAMK2A	PKAC-α	
CSNK2A2	PLK1	
ERK1	SRPK1	
GAK		
	VPS34	Lipid Kinase



- Under-studied and well-studied kinases
- Kinases that are common off-targets
- Balanced representation across phylogenetic tree
- Representation across 7/8 kinase groups

Summary

Novel cellular target engagement assay platform based on EFC

- Improves upon and complements existing cellular target engagement methods
- Simple, quantitative method with no custom chemical tracer or antibody requirements
- Proof of concept demonstrated for several diverse target classes and protein kinases
- Serves major unmet need for cellular target engagment assays

Applications

- Potency rank ordering during lead optimization and HTS hit validation
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- Ideal for targets difficult to test using functional assays
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Learn more about the InCELL Target Engagement assays by visiting discoverx.com/incell and customer development services for developing assays for new target by contacting customdevelopment@eurofins.com.