CARNA BIOSCIENCES

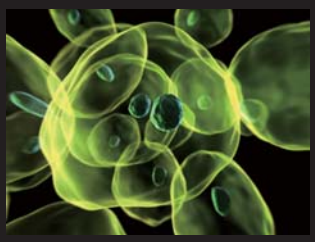
ClariCELL™ Kinase Cell-Based Assay Services

Cell Assay Innovations

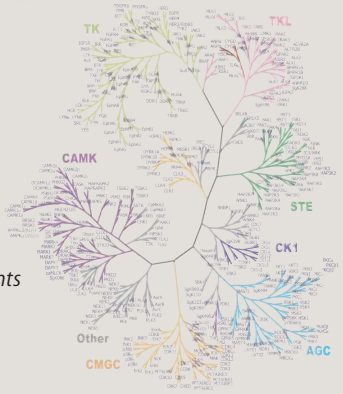
Simplifying Cell-Based Screening and Assay Development

ClariCELL™ Kinase Assay Platform

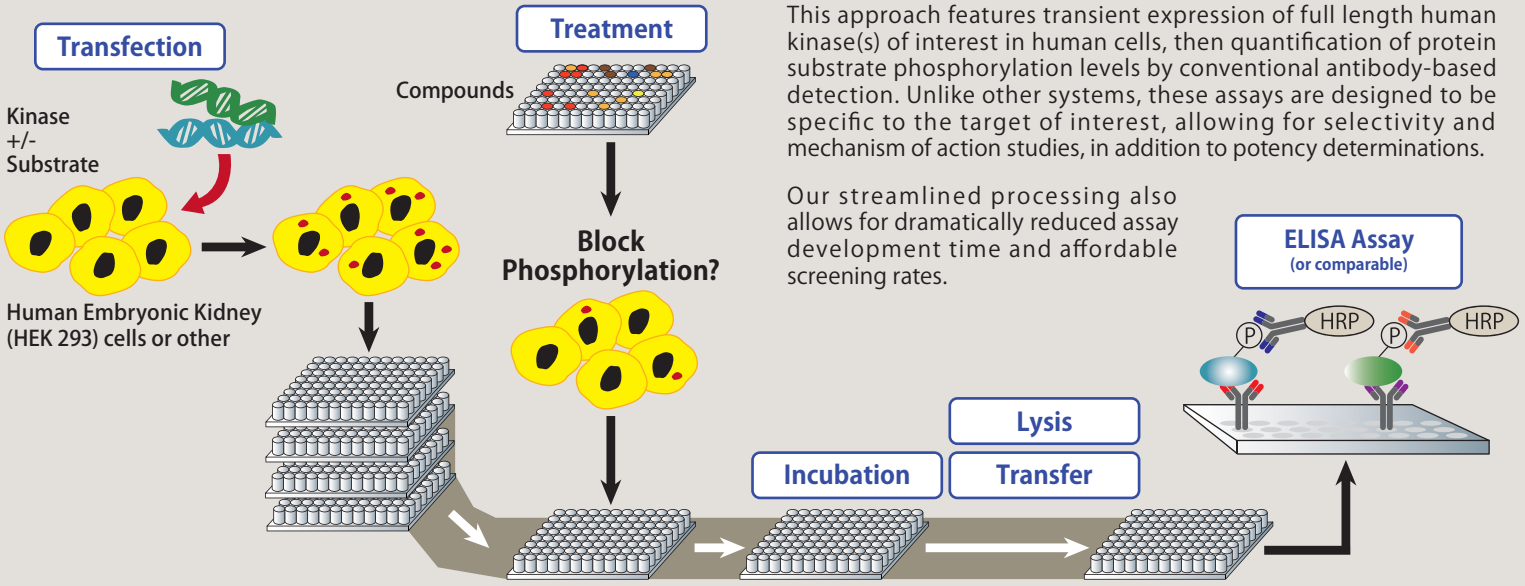
Why CAI's ClariCELL™?



- **Cell-Based Assays for Serine/Threonine and Tyrosine Kinases**
- **Direct Detection of Phosphorylation Events**
  Useful for primary screening, lead optimization, and selectivity profiling
- **Physiologically Relevant Cells Expressing Full Length Kinases**
- **Multiplexing Capability**
  Simultaneous measurement of targets of interest, including drug-resistant mutants
- **Flexibility**
  Choice of kinase(s) and cells of interest



Principle & Method of ClariCELL™ Kinase Cell-Based Assays



This approach features transient expression of full length human kinase(s) of interest in human cells, then quantification of protein substrate phosphorylation levels by conventional antibody-based detection. Unlike other systems, these assays are designed to be specific to the target of interest, allowing for selectivity and mechanism of action studies, in addition to potency determinations.

Our streamlined processing also allows for dramatically reduced assay development time and affordable screening rates.

CAI ClariCELL™ Kinase Cell-Based Assays & Kits

Tyrosine Kinases		Serine/Threonine Kinases	Under Development
ABL1	EphB4	AKT1	AKT [W80A]
ABL1 [T315I]	FGFR1	PDK1	BTK [T474A]
ABL1 [E255V]	JAK3	PIM1	JAK1
BTK	KDR (VEGFR2) NEW	PIM2	JAK2
BTK [C481S] NEW	LCK	PIM3	MST1
	TEC NEW		TYK2

Now **ClariCELL™ assays kits** are available for each target upon request with 4 week turnaround time!

: ClariCELL™ Assay Kit Currently Available

Custom Assay Development Services are also available as well for nearly any kinase/ protein substrate combination of interest, in singleplex or multiplex formats.

Updated : 2013/11/1

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ClariCELL™ Kinase Cell-Based Assay Services

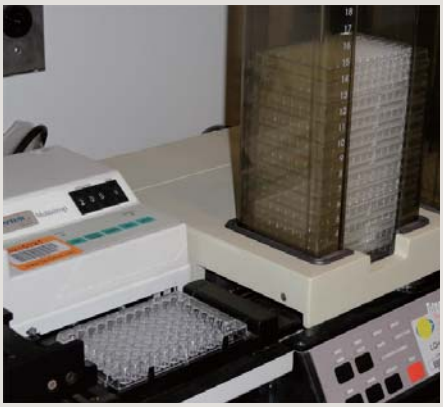
Example of Assay Validation and Study

Cell Assay Innovations

ClariCELL™ FGFR1 Kinase Assay Service

Description

The **ClariCELL™ FGFR1 Kinase Assay** quantifies autophosphorylation of human full-length FGFR1 in human cells. The assay is useful to determine potencies of small-molecule inhibitors against the specified kinase in the context of a cellular environment. Compound testing services are available utilizing the assay.



Overview

Human Embryonic Kidney (HEK 293) cells transiently expressing sequence verified human full-length FGFR1 are exposed to test compound or control, then lysed to release cellular proteins. FGFR1 is captured onto an assay plate, and the extent of autophosphorylation is quantified by ELISA using an antibody specific for the phosphorylation event. Cells expressing kinase deficient FGFR1 [K512M] are also utilized as controls to calculate the % inhibition of test compounds.

4-Step Assay Validation

FGFR1 Expression in Cells

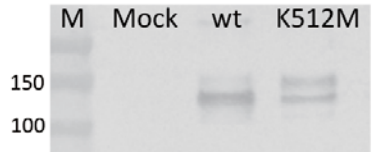


Figure 1: Wild type (wt) or kinase dead (K512M) FGFR1 was expressed transiently in 293 cells. Following cell lysis, an IP Western was performed with appropriate antibodies to capture and detect total FGFR1 protein.

FGFR1 Autophosphorylation in Cells

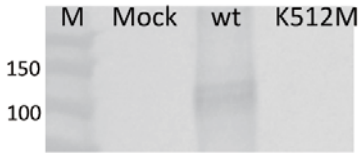


Figure 2: Wild type (wt) or kinase dead (K512M) FGFR1 was expressed transiently in 293 cells. An IP Western was performed with appropriate antibodies to capture and detect phospho- FGFR1 protein.

Quantification of Phosphorylation

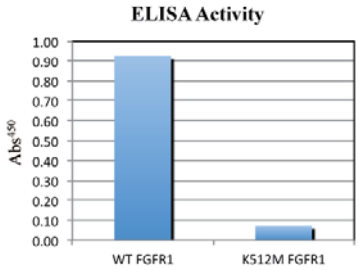


Figure 3: Wild type (wt) or kinase dead (K512M) FGFR1 was expressed transiently in 293 cells. Following cell lysis, an ELISA was performed to quantify the extent of autophosphorylation of FGFR1.

Reference Inhibitor Data

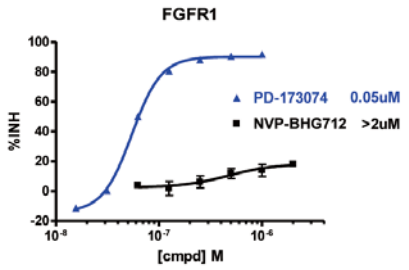


Figure 4: An autophosphorylation assay was performed in the presence of PD-173074, an FGFR1 inhibitor, and NVP-BHG712, a compound that is not expected to inhibit FGFR1. % inhibition data were plotted to determine EC_{50} values.