Cell-Based Tyrosine Kinase Assay Panel

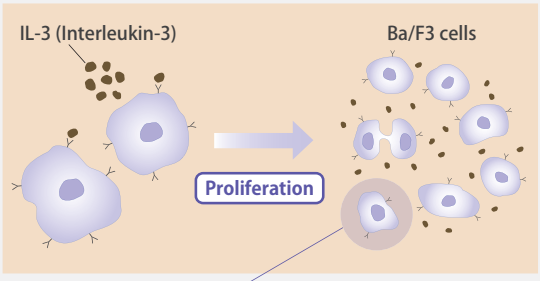
CARNA BIOSCIENCES collaboration with ACD ADVANCED CELLULAR DYNAMICS

Largest Commercially Available Panel of Tyrosine Kinase Cell-Based Assays

Why ACD?

- Comparative cell-based analysis
- To discover direct inhibitory activity to targeted kinases
- Ready-to-run **80** Tyrosine Kinase (TK) Panel
- Time & cost saving solution for your in-house cellular assays

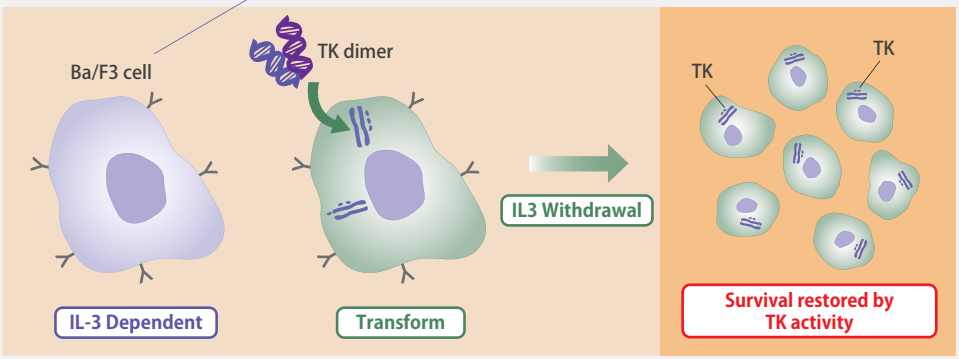
Principle & Method of ACD Cell-Based Assays



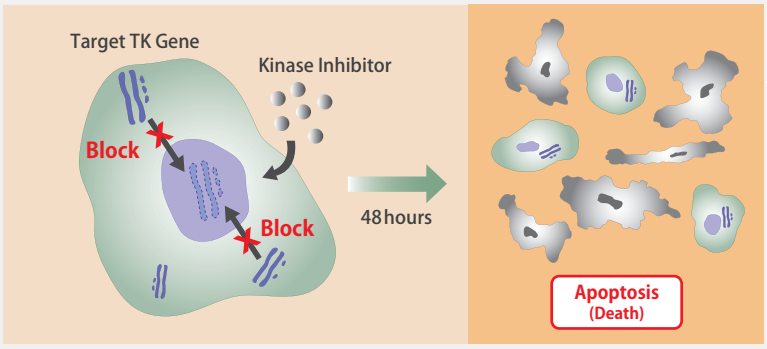
The assay principle builds upon the work of Daley & Baltimore (1988)*.

In this system, IL3-dependent Ba/F3 cells are modified to express an activated recombinant kinase. Following removal of IL3, the modified cells are dependent on the activity of the recombinant kinase for survival and proliferation.

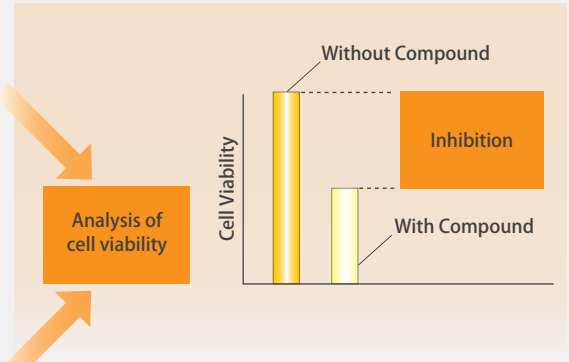
* Daley and Baltimore; Proc. Natl. Acad. Sci. USA. 1988; 85(23):9312-6



Ba/F3 cells are transformed by inducting target kinase dimerization via viral vectors. Activity of the transformed kinase overrides IL3 dependency for cellular proliferation and survival - modified cells no longer require IL3 for growth.



If the kinase inhibitor (compound) specifically blocks the activity of the recombinant kinase, the modified cells undergo programmed cell death (apoptosis).



Each assay is engineered to be dependent upon maintenance of the introduced kinase activity for survival. Inhibition of this activity results in a directly proportional decrease in cell viability.

About ACD

Advanced Cellular Dynamics (San Diego, CA USA) is a leading provider of cell-based assay panel technologies and services to the life-sciences community. ACD develops and deploys families of cell-based screening assays, encompassing broad representations of important target gene families. Their assays are designed to simplify high-throughput screening and profiling of chemical entities in a physiologically relevant cellular environment.

Visit our website for more information:
www.carnabio.com

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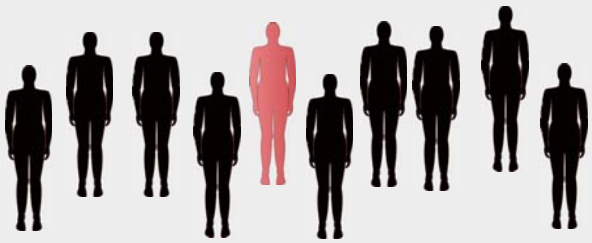
CARNA BIOSCIENCES

ACD's Cell-Based Tyrosine Kinase Assay Panel

Don't miss important biology using traditional assays.

1 EGFR and Lung Cancer

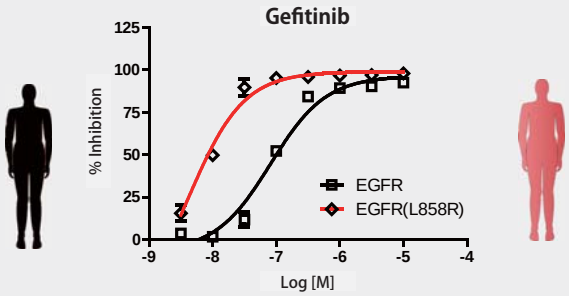
Gefitinib (Iressa™) was the first EGFR tyrosine kinase inhibitor for the treatment of Non-Small Cell Lung Cancer (NSCLC).



➤ Only 10% of the treated population responded.

2 Mutant EGF Receptors

Responding NSCLC patients possess a mutant EGFR.

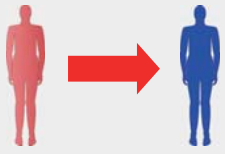


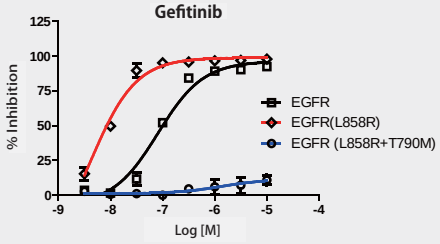
➤ Two "classical" mutations
L858R and **Δ746-750**

➤ Mutant receptors are much more responsive to Gefitinib.

3 Gefitinib Resistance

Responsive patients become resistant over time.





➤ Resistance due to secondary "gatekeeper" mutation (T790M).

➤ Double mutant receptors (L858R + T790M) are much less responsive to Gefitinib.

4 Why Use Cell-based Assays?

Kinase biology can be complex.

Biochemical Assay*

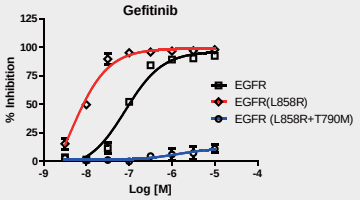
Kinase	K_d, nM		$K_d/K_{m(ATP)}$, x 10^{-3}	
	Gefitinib	AEE788	Gefitinib	AEE788
WT	35.3 ± 0.4	5.3 ± 0.3	6.8	1
T790M	4.6 ± 0.1	27.6 ± 0.7	0.78	4.7
L858R	2.4 ± 0.1	1.1 ± 0.1	0.016	0.0074
L858R/T790M	10.9 ± 0.6	18.6 ± 0.5	1.3	2.2

The ratio $K_d/K_{m(ATP)}$ provides a relative estimate of inhibitor potency.

Notice that L858R/T790M affinity for Gefitinib is reduced < 5-fold relative to L858R.
In tumors, the response differs by > 100-fold!

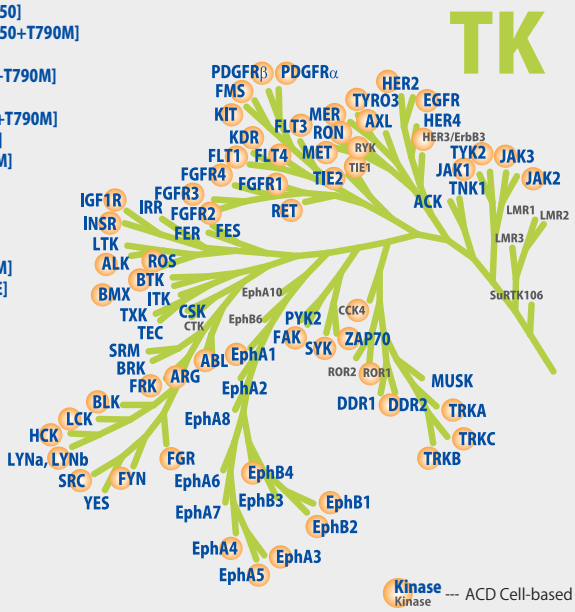
* Adapted from Yun et. al. (2008). Proc. Natl. Acad. Sci. 105: 2070

ACD Cell-Based Assay



➤ Tumors bearing L858R/T790M respond poorly to Gefitinib. These differences can be missed when evaluation is performed using traditional biochemical assays, but are captured using ACD cell- based assays.

ALK [L1196M]
EGFR [Δ746-750]
EGFR [Δ746-750+T790M]
EGFR [L858R]
EGFR [L858R+T790M]
EGFR [L861Q]
EGFR [L861Q+T790M]
EGFR [T790M]
FGFR1 [V561M]
FGFR2 [K660E]
FGFR2 [K660N]
FGFR2 [N550K]
FGFR2 [V565I]
FGFR3 [K650M]
FGFR4 [V550E]
KIT [D816V]
KIT [K642E]
KIT [N822H]
KIT [T670I]
KIT [V654A]



ACD Cell-Based TK Assays Available for Screening Services

80 Total Kinases - Broad Coverage of the Tyrosine Kinome!

ABL(BCR-ABL)
ALK
ALK [L1196M]
ARG(ABL2)
AXL
BLK
BMX
BTK
CCK4(PTK7)
DDR2
EGFR
EGFR [Δ746-750]
EGFR [Δ746-750+T790M]
EGFR [L858R]
EGFR [L858R+T790M]
EGFR [L861Q]
EGFR [L861Q+T790M]
EGFR [T790M]
EphA1
EphA3
EphA4
EphA5
EphB1
EphB2
EphB4
FAK
FGFR1
FGFR1 [V561M]
FGFR2
FGFR2 [K660E]
FGFR2 [K660N]
FGFR2 [N550K]
FGFR2 [V565I]
FGFR3
FGFR3 [K650M]
FGFR4
FGFR4 [V550E]
FGR
FLT1
FLT3
FLT4
FMS(CSF1R)
FRK
FYN
HCK
HER2(ERBB2)
HER3(ERBB3)
IGF1R
INSR
JAK1
JAK2
JAK3
KDR
KIT
KIT [D816V]
KIT [K642E]
KIT [N822H]
KIT [T670I]
KIT [V654A]
LCK
LYN
MER(MERTK)
MET
PDGFRa
PDGFRb
RET
RON(MST1R)
ROR1
ROS(ROS1)
RYK
SRC
SYK
TIE1
TIE2
TRKA(NTRK1)
TRKB(NTRK2)
TRKC(NTRK3)
TYK2
TYRO3
ZAP70

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