KiNativ™ Application Note

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ATPase Profiling

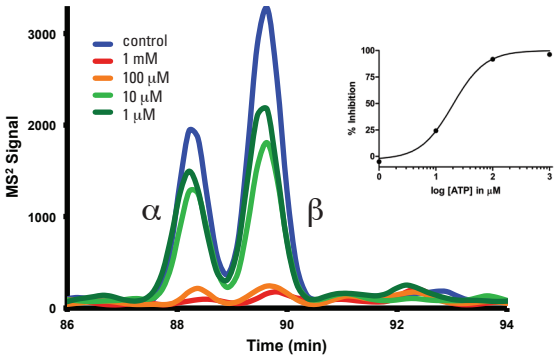
Active site profiling of ATPases and related nucleotide binding proteins

The human genome contains hundreds of ATP-dependent enzymes that represent potential drug targets. ActivX Biosciences' KiNativ™ platform has been recently expanded to provide for the profiling of approximately 180 of these enzymes. The technology is identical to the well-validated methodology used to profile protein kinases. An ATP acyl-phosphate probe is used to label the active sites of enzymes that bind ATP (or related nucleotides, NAD, FAD, SAM, etc.) directly in native cell lysates or tissues. Because labeling occurs within the active site, the relative amount of labeling is sensitive to the binding of competitive inhibitors. The relative potency and target specificity of ATP-competitive compounds against hundreds of ATPases can be assessed in a single experiment. This initial offering is being continually refined in order to cover a larger number of ATPases. Please contact us to inquire about your enzyme of interest.

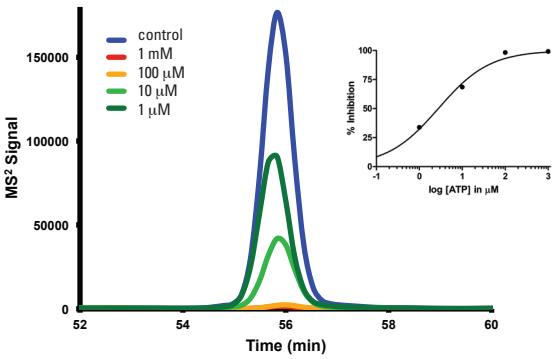
Enzyme Class	# profiled members
HSP90	4
HSP70	9
HSP60 (Chaperonin)	9
AAA+ (ABC transporters)	17
AAA+ (Proteasomal ATPase)	6
AAA+ (Other)	29
Ubiquitin-activating E1	6
ATP-Grasp domain	9
ATP-dependent helicase	14
Small molecule kinase	17
Nudix hydrolase	4
Aminoacyl-tRNA synthetase	8
Actin	6
Miscellaneous	40

Labeling site validation

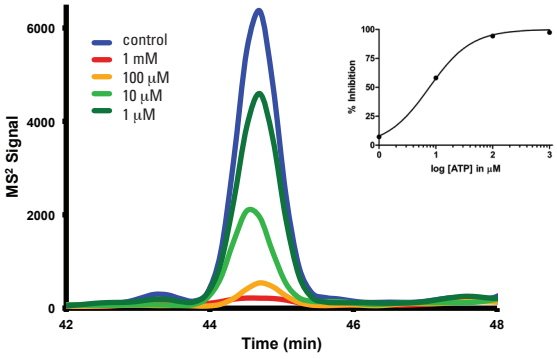
HSP90α, HSP90β



p97 ATPase



HSP70-1a



Enzyme Name	ATP concentration 1 mM	100 µM	10 µM	1 µM	ATP IC_{50} (µM)
HSP90α, HSP90β	96.3	91.8	24.2	-5.0	19.8
HSP70-1a	97.4	94.3	58.1	7.2	7.6
p97 ATPase (ATP site1)	99.2	98.3	68.5	33.9	2.8

values in % inhibition

Labeling sites for the ATP probe were validated to be active site peptides by performing competition experiments with free ATP. Using this criteria, over 240 labeling sites from approximately 180 proteins have been validated.

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Profiling Heat Shock Protein Inhibitors

Enzyme Name	Geldanamycin 20μM	2μM	200nM	20nM	17-AAG 20μM	2μM	200nM	20nM	Radicicol 10μM	1μM	100nM	10nM
HSP90α, HSP90β	98.5	96.3	45	-0.2	94.2	56.2	3.3	-5.3	98.8	98.7	28.1	1.6
HSP90α, HSP90β	96.9	95.9	50.4	5.1	95.5	73.4	10.2	-8.7	98.6	98.1	27.4	-10.1
Endoplasmin (TRA1, GRP94)	93.4	60.6	4.9	8.7	71.7	14.1	-7	-27.8	99.3	98.2	14.2	-2.4
TRAP1 (HSP75)	73.6	14.6	-5.8	-1	10.4	-1.4	-10.1	-10.9	95.3	90.7	-1.7	-0.1

Enzyme Name	BIIB021 10μM	1μM	100nM	10nM	NVP-AUY922 10μM	1μM	100nM	10nM
HSP90α, HSP90β	98.8	98.5	14.5	-16.3	99.2	98.8	37.4	9
HSP90α, HSP90β	98.4	97.8	31.1	-7.1	98.7	97.6	29.3	6
Endoplasmin (TRA1, GRP94)	91.1	24.3	-24.2	-16.1	98.4	86.7	-8.6	-23.3
TRAP1 (HSP75)	94	64.6	-5.8	-0.1	95.3	85.7	0	-7.9

The abundance of HSP90 isoforms in a typical cell lysate preparation is > 100 nM, thus any inhibitor with an affinity of at least 100 nM will titrate the total enzyme in a typical profiling experiment. Shown values were obtained in non-reducing conditions.

The profiling of HSP90 inhibitors revealed no inhibition outside of the 4 HSP90 family members. However, profiling of the HSP70 inhibitor, VER-155008, showed off-target inhibition of 5 nucleotide-binding proteins in addition to inhibition of 5 HSP70 isoforms. All compounds were profiled against ~ 160 ATPases found in HL60 cell lysates.

Enzyme Name	VER-155008 100uM	10uM	1uM	0.1uM	IC_{50}
HSP70-1a	94.2	86.7	58.6	19	0.66
GRP78	89.4	77.3	44.3	19	1.4
HSC70	95.4	84	53.8	16.1	0.86
HSP70-13 (STCH)	63.1	19.1	17.6	0.6	57.2
GRP75	57.3	12.2	4	-13.8	64.5
Deoxycytidine kinase	94.7	70.1	9.4	-19.5	4.2
Adenosylhomocysteinase	68.1	18.7	4.3	-7.8	41.7
IPP isomerase 1, IPP isomerase 2	48.8	15.5	17.1	3.7	139
8-oxo-dGTPase	43.2	8.3	-13.9	0.6	120
Farnesyl pyrophosphate synthetase	50.6	-2.2	-7.1	-16.6	87.9

Profiling Biologically-Active Flavonoids

Enzyme Name	Quercetin	Myricetin	Epigallocatechin gallate
8-oxo-dGTPase	1.2	12	77
Ap4A hydrolase	4.6	43	36
IPP isomerase 1, IPP isomerase 2	2.6	30	> 150
CoenzymeA diphosphatase	1.6	53	> 150
Mevalonate kinase	4.9	35	23
Deoxycytidine kinase	34	117	97
NDP kinase 3	22	105	> 150
Cytidylate kinase	97	98	83
Thymidine kinase 2	120	60	47
Farnesyl pyrophosphate synthetase	1.7	13.7	28
2',5'-phosphodiesterase 12	2.0	4.2	> 150
Amidophosphoribosyltransferase	8.6	> 150	> 150
SAICAR synthetase	1.8	50	> 150
tRNA-dihydrouridine synthase	87	3.7	6.3
DNA-directed RNA Pol III	38	126	> 150
dCTP pyrophosphatase	8.0	13	28
HPRTase	> 150	70	33
AMPKγ1 (site 2)	91	100	42
AMPKγ1, AMPKγ2 (site 1)	> 150	> 150	> 150
AMPKγ2 (site 2)	72	> 150	61
Autophagy-related protein 7	> 150	77	37
Ubiquitin-activating enzyme E1	> 150	> 150	100
NEDD8-activating enzyme E1	> 150	62	> 150
ATP-binding cassette sub-family D3	> 150	59	87
AAA domain-containing protein 1	34	> 150	51
KIAA0564	25	8.1	6.2

Legend	IC_{50} in μM
	< 5 μM
	5 - 25 μM
	25 - 75 μM
	75 - 150 μM
	> 150 μM

Quercetin and myricetin are naturally occurring flavonoids found in many plants. They are thought to have antioxidant, anti-inflammatory and anti-cancer properties through a variety of mechanisms, including ATP-competitive inhibition of lipid and protein kinases. EGCG is a related flavonoid and the major bio-active ingredient of green tea. The ATPase profiling results demonstrate that they have affinity for a variety of enzyme classes.

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