

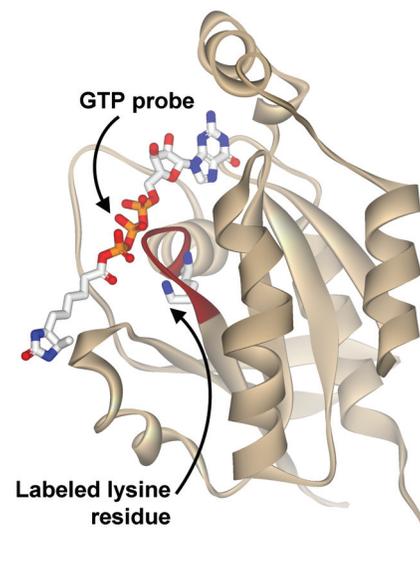
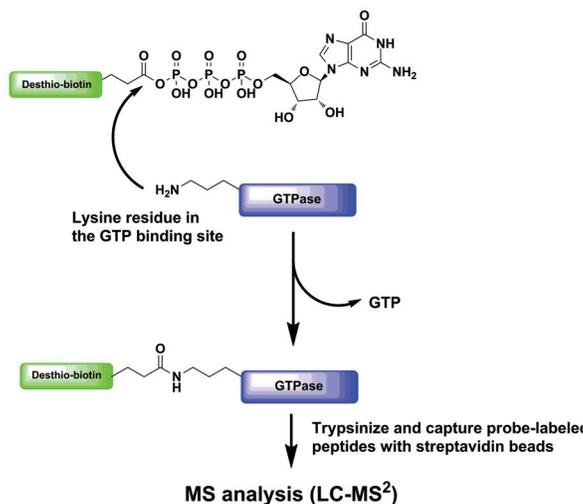
# GTPase Profiling

## Active site profiling of native GTPases

GTPases comprise a diverse family of enzymes that participate in a variety of cellular processes including signal transduction, protein synthesis, and intracellular transport. Importantly, the misregulation of a number of GTPases is known to be associated with cancers.

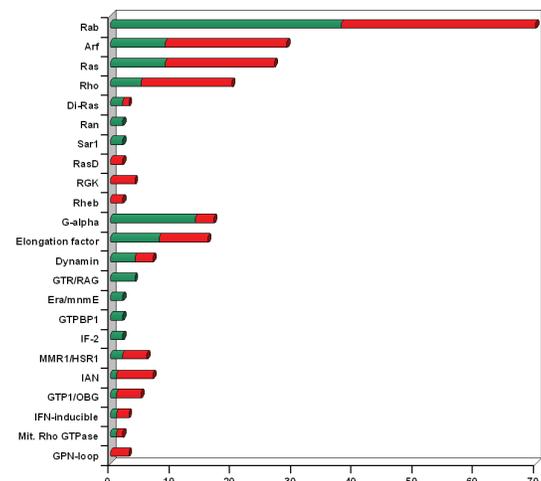
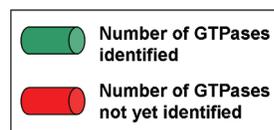
ActivX has developed a desthiobiotin acyl phosphate GTP probe that covalently modifies GTPases in the nucleotide binding site. Thus far, enzymes from most subfamilies of GTPases have been identified, suggesting that the probe could be a useful tool for profiling the distribution of GTPases in various proteomes.

The GTP acylphosphate probe covalently modifies a conserved lysine in the phosphate binding loop (illustrated as a dark red ribbon) present in all GTPases.



## Current GTPase coverage

The GTP probe was used to identify GTPases in a variety of human and mouse proteomes. For each family of GTPases, the number of GTPases identified is shown in green with the remainder of known family members shown in red.



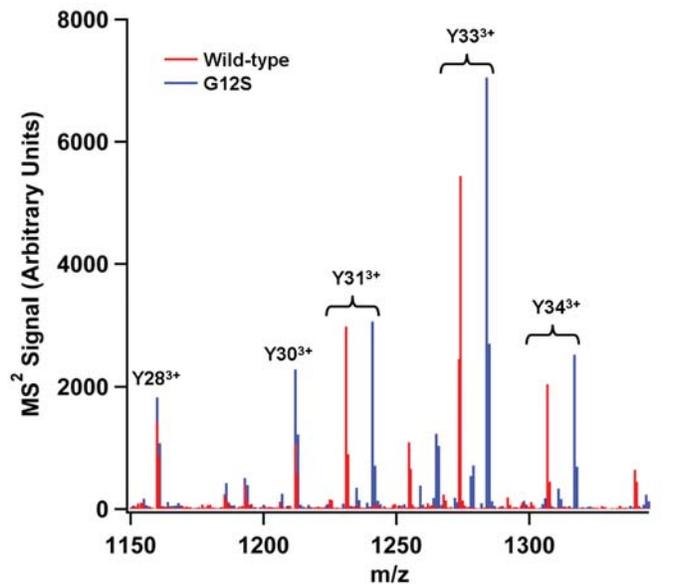
For more information about ActivX technologies call 858-526-2515 or visit [www.activxprobes.com](http://www.activxprobes.com)

# GTPase Profiling

Active site profiling of native GTPases

## Identification of mutant RAS (H, K, N)

Mutations in RAS isoforms (H,K,N) at codons 12 and 13 (G→A,S,C,V and D) result in resistance to GAP-stimulated GTP hydrolysis leading to constitutively active RAS signaling. These mutations have been identified in a number of human cancers. The fact that the probe-labeled peptide for RAS contains the codons 12 and 13 permits the identification and quantification of mutant RAS proteins using the GTP acylphosphate probe.



Parent ion 6-LVVVGAG(S)GVGK\*SALTIQLIQNHVDEYDPTIEDSYRK-41

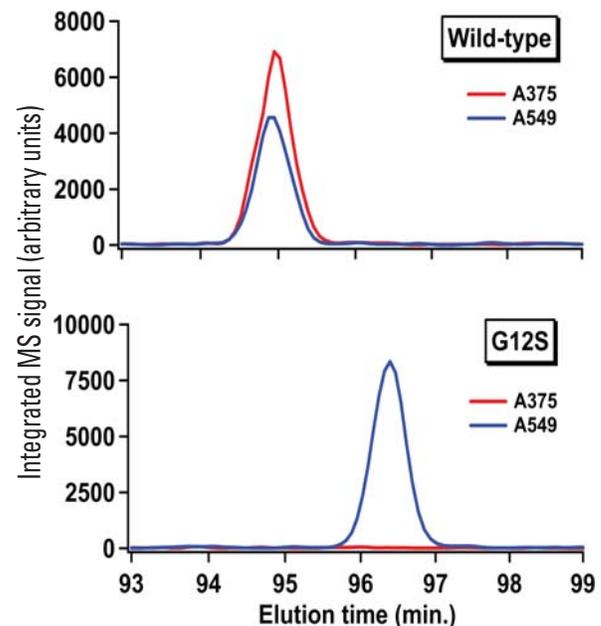
Fragments {

- Y34: VGAG(S)GVGK\*SALTIQLIQNHVDEYDPTIEDSYRK
- Y33: GAG(S)GVGK\*SALTIQLIQNHVDEYDPTIEDSYRK
- Y31: G(S)GVGK\*SALTIQLIQNHVDEYDPTIEDSYRK
- Y30: GVGK\*SALTIQLIQNHVDEYDPTIEDSYRK
- Y28: GK\*SALTIQLIQNHVDEYDPTIEDSYRK

## Integrated MS<sup>2</sup> signal for WT and G12S RAS

A375: wild-type H, K, N RAS

A549: wild-type H, N RAS, G12S K RAS



## Probe labeling of GTPases in the presence or absence of EDTA

GTPases tightly bind GTP and GDP in the presence of Mg<sup>2+</sup>. The chelation of Mg<sup>2+</sup> by EDTA significantly weakens the affinity of the nucleotide, allowing for nucleotide exchange. When lysates were labeled with the GTP probe in the absence or presence of EDTA multiple GTPases exhibit a differential response to EDTA exchange. While the labeling of some GTPases is strongly stimulated by EDTA, other GTPases label equally well with or without EDTA exchange. These differences may reflect different activity states of the GTPases in the lysate.

Fold-change in MS signal upon EDTA exchange (selected GTPases)

Family	GTPase	HL60	PANC1	HEL	KARPAS
Small GTPase	(ARF1,3,4,5)	-1.09	-1.07	-1.25	-1.28
	ARL1	-1.09	1.47	-1.52	-1.47
	RAN	1.72	-1.52	-1.11	1.72
	(RAB8A,10)	1.79	3.16	1.79	1.39
	(RAB11A,B)	1.46	2.36	2.13	2.72
	RAB14	1.29	1.44	1.35	1.6
	RAB18	2.05	2.65	1.56	1.97
	(RAB1A,B,C)	2.6	3.22	3.25	5
	RAB21	2.83	2.24	3	3.18
	RAB35	3.28	4.88	3.33	3.61
	(RAB6A,B)	1.41	1.82	1.18	-1.01
	RAB7A	3.91	6.93	5.16	4.57
	(RhoA,C)	2.85	0.79	3.7	3.8
	CDC42	1.85	2.64	2.49	2.61
	(RAS H,K,N)	19.3	9.38	5.21	7.88
RALA,B	1.42	1.45	2.54	1.88	
RAP1A,B	3.17	3.55	1.57	2.98	
G-alpha	GNAT1,2,3	1.1	-1.06	-1.09	-1.11
EF-Tu/EF-1A	(EEF1A1,2,3)	-1.15	-1.33	-1.08	-1.45
	TUFM	-1.39	1.09	-1.54	-1.59
GTP Binding Protein	GTPBP1	-1.18	-1.22	-1.52	-1.3
	GTPBP2	-1.04	1.03	-1.49	-1.59

8 fold increase  
4 - 8 fold increase  
2 - 4 fold increase  
No change



For more information about ActivX technologies call 858-526-2515 or visit [www.activxprobes.com](http://www.activxprobes.com)