

Rab5 Affinity Chromatography without Nonhydrolyzable GTP Analogues

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Rab5 is an important small GTPase involved in endocytosis and membrane trafficking. Rab5-binding proteins can be identified using Rab5 affinity chromatography with nonhydrolyzable GTP analogues such as GTP S or GppNHp. However, this method requires significant quantities of the GTP analogue and is thus time-consuming and expensive. In the present report we show a faster and more cost-effective method that does not use a GTP analogue but uses constitutively the active Rab5 mutant (Rab5Q79L) as a ligand. To validate this method, the binding of EEA-1 was confirmed and several novel Rab5-binding proteins were also identified by 2-dimensional electrophoresis and liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS).

Key words: GTP S, GppNHp, Rab5 Affinity Chromatography