University of Dundee

Contact:- Dr C. James Hastie

Antibody Datasheet

Product description:- Anti-Rab13 phospho Ser 111

Antigen:- KSIKENAS*AGVERLR [residues 104 - 117 of human]

Sheep Number:- S505D

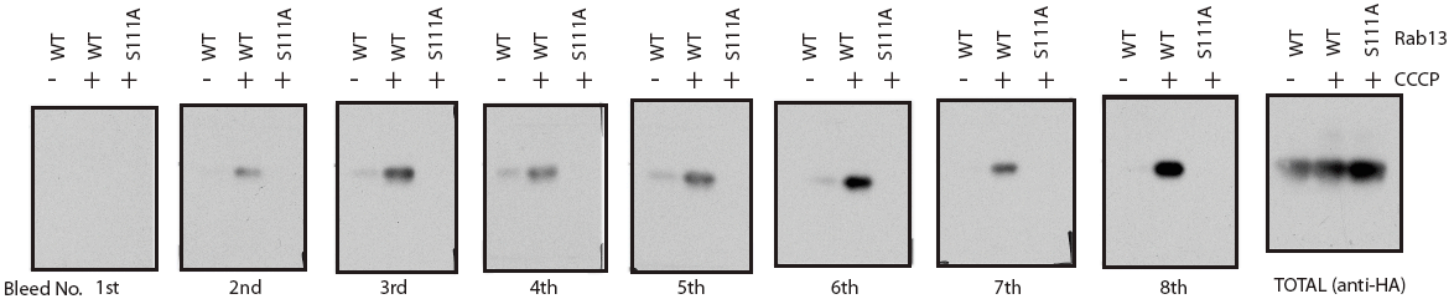
Formulation:- Phosphate Buffered Saline

Storage temperature:- –20 °C

Purification Method:- Affinity purified against phospho specific peptide

Working Concentration:-
For immunoblotting use at 0.2 ug/ml plus 2 ug/ml of non-phospho peptide
Please note that Rab13 needs to be immunoprecipitated first before immunoblotting.

Immunoblotting:



HA-Rab13 was transiently transfected into Flp In HEK293 stable cell lines expressing PINK1-Flag tagged Wildtype (WT). Cells were induced for PINK1 expression with doxycyclin for 24 hrs and stimulated with and without CCCP (mitochondrial depolarizing agent) for 3 hours. HA-Rab13 S111A mutant was transfected into PINK1-Flag WT stable cell lines. Cells were induced for PINK1 expression with doxycyclin for 24 hours and stimulated with CCCP (mitochondrial depolarizing agent) for 3 hours. 1 mg of whole cell lysate was immunoprecipitated for Rab13 using 10 ul of HA-agarose affinity resin. Proteins were resolved by SDS-PAGE and subjected to western blot analysis. The blot was probed with the bleeds of anti-Rab13 phospho Ser 111 S505D antibody as indicated.

Antibody recognises HA-Rab13 when it is expressed in cells stably expressing PINK1 under conditions of mitochondrial depolarization and not under basal conditions (A faint band is observed under basal conditions which could be unphosphorylated Rab13 being picked up unspecifically). Also the specificity of the antibody is confirmed with loss of recognition of HA-Rab13 S111A mutant.