

### Antibody Quality Control of Phospho Antibodies:

Name of Antibody: PINK1 phospho Thr 257

Sheep Number: S114D

Bleed Number: 3<sup>rd</sup> bleed is the best

**Publication reference : Unpublished data – hence please treat as highly confidential**

### Immuno Blotting:

Does not recognize endogenous or recombinant protein upon immunoblotting whole cell lysates/mitochondrial fractions .

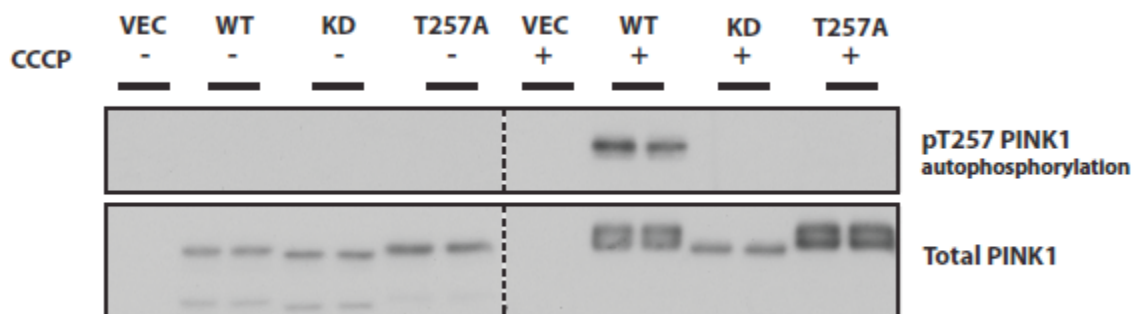
Does it recognise endogenous protein without immunoprecipitation: NO

[Please state tissues or cell lines and stimuli used]

### Immuno blotting after Immunoprecipitation:

Does it immuno blot only after immunoprecipitation: Yes

Flp In HEK293 stable cell lines expressing either Flag alone, PINK1-Flag Wildtype (WT), PINK1-Flag Kinase dead (KD) or phospho dead mutant PINK1- flag T257A, were induced for expression with doxycyclin for 24 hrs. The cells were stimulated with either DMSO or CCCP for 3hrs and subjected to mitochondrial fractionation. 0.5mg of mitochondrial extracts were immunoprecipitated with 5ul of anti-flag agarose beads to immunoprecipitate recombinant PINK1. Proteins were resolved by 8% SDS-PAGE and subjected to western blot analysis. Proteins were probed with S114D phospho T257 antibody (3<sup>rd</sup> bleed). The antibody recognises mitochondrial Wild-type PINK1 only upon CCCP stimulation. It does not recognise PINK1 KD. Moreover, the specificity of the antibody is confirmed by loss of recognition with the phospho dead T257A mutant.



**Concentration used for immuno blotting:** 1ug/ml (with 10ug/ml of dephos peptide)