

Antibody Quality Control of non-phospho antibodies:

Name of Antibody: PINK (112-496 mouse)

Sheep Number: S857C

Bleed Number: 1st bleed, 2nd bleed, 3rd bleed - immunoblotting
3rd bleed - Immunoprecipitation

Date Purified: 19.5.10

Immuno Blotting:

Can recognise recombinant mouse PINK1 protein expressed in HEK 293 cell lines

Does not immunoblot endogenous PINK1 in mouse embryonic fibroblasts

Concentration used: 1ug/ml

Immunoprecipitation:

Can immunoprecipitate recombinant mouse PINK1 protein expressed in HEK 293 cell lines.

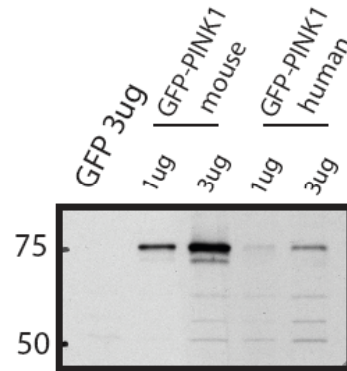
Still being evaluated whether immunoprecipitates endogenous protein.

Amount used:

For immunoprecipitation use 5 ug of antibody per 1 mg of cellular extract

HEK293 cells were either transfected with GFP empty vector/ GFP-PINK1 (125-end) mouse/ GFP-PINK1 (125-end) human and 1 and 3µg of protein was subjected to SDS-PAGE analysis before being transferred to nitrocellulose membrane and immunoblotted with anti-PINK1 (235-end) at 1 µg/ml overnight at 4°C.

Binding of the primary antibody was detected using rabbit peroxidase conjugated anti-sheep IgG antibody (1 in 2500 dilution) and enhanced chemiluminescence.



S857C 3rd bleed

PINK1 was immunoprecipitated from 1mg HEK 293 cellular extracts over-expressing mouse GFP-PINK1 (125-END). Immunoprecipitation were performed using 5ug of pre-immune serum covalently coupled to protein G sepharose (lane 1) ; 5ug anti-PINK1 S774C antibody covalently coupled to protein G sepharose (lane 2). Immunoprecipitates were subjected to SDS-PAGE analysis before being transferred to nitrocellulose membrane and immunoblotted with anti-PINK1 S774C antibody at 1 μ g/ml overnight at 4°C. Binding of the primary antibody was detected using rabbit peroxidase conjugated anti- sheep IgG antibody (1 in 2500 dilution) and enhanced chemiluminescence.

