

Antibody Quality Control of non-phospho antibodies:

Name of Antibody: ALPK1[395-823]

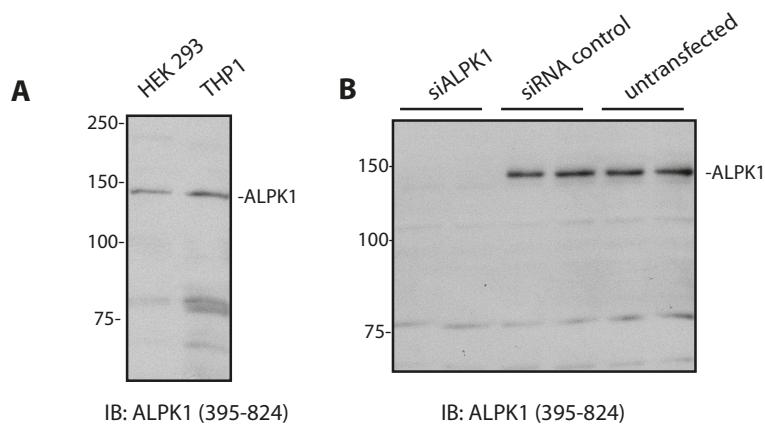
Sheep Number: S593D

Bleed Number: 5th (bleeds 1-4 also work in immunoblotting but not as well as the 5th bleed)

Date Purified:

Immuno-blotting:

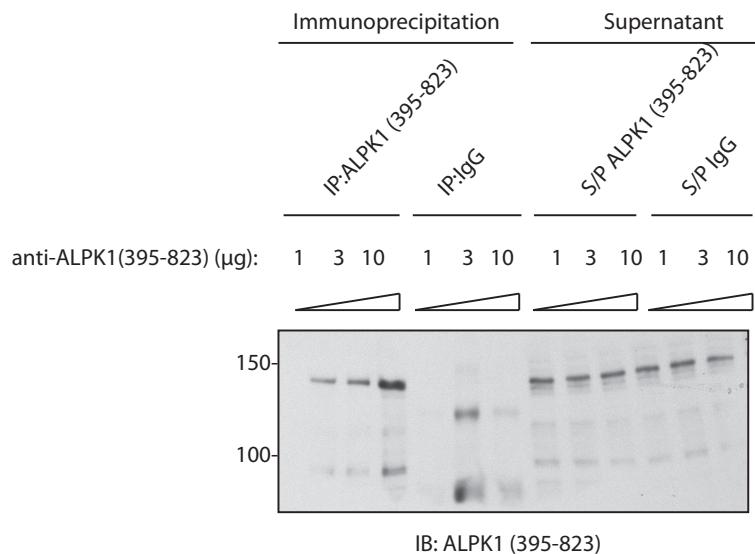
Does it recognise endogenous protein without immunoprecipitation: Yes (human THP1 monocytes and HEK 293 cells were used)



Immunoblotting of ALPK1. **A.** HEK293 or THP1 cell extracts (20 µg protein) were subjected to SDS-PAGE on 6% polyacrylamide gels and immunoblotted with 1.0 µg/ml of the ALPK1 antibody raised against amino acid residues 395-824 of ALPK1. The antibody was diluted in **B.** Same as A, except that the HEK293 cells were transfected with an ALPK1 siRNA or control siRNA prior to cell lysis. After siRNA knock-down of ALPK1, the 140 kDa band could no longer be detected by the antibody showing that the 140 kDa band was indeed ALPK1.

Immunoprecipitation:

Does it immunoprecipitate endogenous protein: Yes (THP1 cell line) but immunodepletion is much poorer than with the antibody raised against amino acid residues 1-358 of ALPK1.



Immunoprecipitation of ALPK1 in cell extracts. Increasing amounts of the antibody raised against amino acid residues 395-823 of ALPK1 or a control IgG were used to immunoprecipitate ALPK1 from 0.5 mg of THP1 cell extract protein in a total volume of 0.5 ml. After immunoprecipitation, the ALPK1 was released from the antibody in SDS, subjected to SDS-PAGE and immunoblotted using the same antibody (first 6 lanes from the left). The supernatant obtained after immunoprecipitation was analysed in a similar manner (lanes 7-12 from the left) to estimate the proportion of ALPK1 that had been immunoprecipitated.