

## Antibody Quality Control of Phospho Antibodies:

Name of Antibody: MYPT1 phospho Ser 554

Sheep Number: S661C

Bleed Number: 1-3

Date Purified: 1<sup>st</sup> bleed 14/05/09  
2<sup>nd</sup> bleed 09/06/09  
3<sup>rd</sup> bleed 09/07/06

## Immuno Blotting:

Does it recognise endogenous protein without immunoprecipitation: NO  
Tested on 20 µg HEK 293 cell lysate. Non stimulated.

Concentration used: 1 µg/ml with 10 µg/ml of dephospho-peptide in 5% milk

## Immuno blotting after Immunoprecipitation: YES

Bleed 3 interacts with overexpressed MYPT1 wt and S554A mutant. Bleeds 1 and 2 are more specific.

Tested on immunoprecipitates of FLAG-MYPT1 from 1 mg of HEK293 cell lysates.

Concentration used for immuno blotting: 1 µg/ml with 10 µg/ml of dephospho-peptide in 5% milk

Antibody and concentration used for immunoprecipitation: 5 µl of FLAG-agarose from Sigma (1/20 of the IP was loaded onto the gel)

