

**Antibody Quality Control of non-phospho antibodies:**

Name of Antibody: Anti-Rub1

Sheep Number: S105D

Bleed Number: 3

Date Purified: 24/5/11

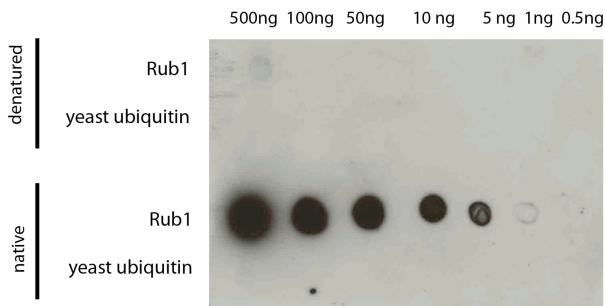
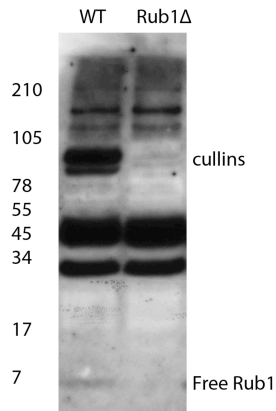
**Immuno Blotting:**

Does it recognise endogenous protein without immunoprecipitation:

Recognizes both Rub1 conjugated to the cullins and endogenous free Rub1. Yeast strains were both S288C background. Also displays specific recognition of Rub1 over the very similar protein ubiquitin.

Concentration used: 1:500 overnight. TBST (later results were more successful for visualising free Rub1 and minimising strength of nonspecific bands when PBST was used.)

anti-Rub1 3rd Bleed raised against residues 21-35



Immunoprecipitation:

Does it immunoprecipitate endogenous protein:

No—two attempts were made but both were unsuccessful. No visible Rub1 or Rub1 conjugated to cullins in the eluent,

Amount used:

(? Notebook 2 handwritten on bottom right of a page In my lab notebook around the 16<sup>th</sup> or 17<sup>th</sup> of this month ?)

Immuno blotting after Immunoprecipitation: Only see light and heavy chain possible extremely faint cullins.

Does it immuno blot only after immunoprecipitation: No

Concentration used for immuno blotting: 1:500 overnight

Antibody and amount used for immunoprecipitation: (? In my lab notebook 2 around the 16<sup>th</sup> or 17<sup>th</sup> of this month ?)