

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

Name of Antibody: antibody raised against **mouse Optineurin amino acid residues 520-531**

Sheep Number: **S685D**

Bleed Number: **4<sup>th</sup>/5<sup>th</sup>**

Date Purified: **20.06.2014 (4<sup>th</sup>)/12.08.2014 (5<sup>th</sup>)**

Immuno Blotting:

Does it recognise endogenous protein without immunoprecipitation: **No.**

Cell line used - **Murine bone marrow derived macrophages.**

Concentration used: **0.5 µg/ml**

Immunoprecipitation:

Does it immunoprecipitate endogenous protein: **Yes**

Cell line used - **Murine bone marrow derived macrophages.**

Amount used: **Optimal immunoprecipitation is obtained using 2 micrograms of antibody for 4 hours. Under these conditions about half the optineurin was immunoprecipitated. The 4<sup>th</sup> and fifth bleeds are equally good – see attached figure. The figure also shows that a second antibody raised against optineurin residues 329-341 does not immunoprecipitate Optineurin. Like the optineurin[520-531] antibody the optineurin[329-341] antibody also fails to recognise optineurin in immunoblotting experiments in cell extracts**

Immuno blotting after Immunoprecipitation:

Does it immuno blot only after immunoprecipitation:

**No., so the optineurin antibody from Abcam was used for immunoblotting.**

Concentration used for immuno blotting: **0.5µg/ml (Abcam)**

Antibody and amount used for immunoprecipitation:

