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

**Name of Antibody**: anti-Miro2 (human)

**Sheep number**:S247D

**Immunogen**: GST-MIRO2 (DU 38256)

**Bleed number:** 1st, 2nd and 3rd – immunoblotting

 3rd bleed - immunoprecipitation

**Immuno Blotting:**

Vector control, Miro1-HA and Miro2-HA were transiently expressed in HEK 293 cells and whole cell lysates were made. 25ug of lysates were used to confirm specificity of the Miro2 antibody. 1ug/ml of anti-Miro2 antibody was used for immunoblotting. We expressed Miro1, an orthologue of Miro2 to see if the antibody can cross-react with Miro1.

The antibody specifically recognized over-expressed Miro2 and also recognized endogenous Miro2. Both endogenous and over-expressed protein ran at 75 kDa and we realized that Miro-HA over-expression is only twice as much as endogenous as we observe a slight increase in band intensity in the Miro2 over-expressed lane. We also used a commercial Miro2 antibody, which detects endogenous protein and we observe a similar scenario. The DSTT antibody was however more sensitive than the commercial Abnova Miro2 antibody.



**Immunoprecipitation**

100ug of whole cell lysates from HEK293 cells was immunoprecipitated for endogenous Miro2 either using 5ug anti-Miro2 antibody covalently coupled to Protein-G sepharose beads or with 5ug pre-immune sheep IgG coupled to protein- G sepharose. 25ug of HEK 293 whole cell lysate was ran as an input control for the immunoprecipitation.

We observe that the Miro2 antibody can specifically pull-down endogenous Miro2 as we do not observe any band in the IgG control and we see a band of the correct molecular weight when the pull down was performed with Miro2 antibody.



**Amount used:**

For immunoprecipitation of endogenous protein 5 ug of antibody per 100u g of cellular extract was used