Western blot analysis and ELISA was carried out, using the methods described below.

1. Western blot

100ng of each antigen was separated on SDS-PAGE, either under reducing or non-reducing conditions. Proteins were transferred using Transblot for 10 minutes at 25V. 2% dry milk in PBS-T was used as blocking buffer and dilution buffer for antibodies. Primary antibodies (1:1,000) are shown in Figure 1 below; anti-mouse-IgG-HRP secondary antibody was used at 1:2,500. All steps were carried out for 1h at room temperature with gentle rocking. TMB Membrane was used for detection. Development time ~40 min.

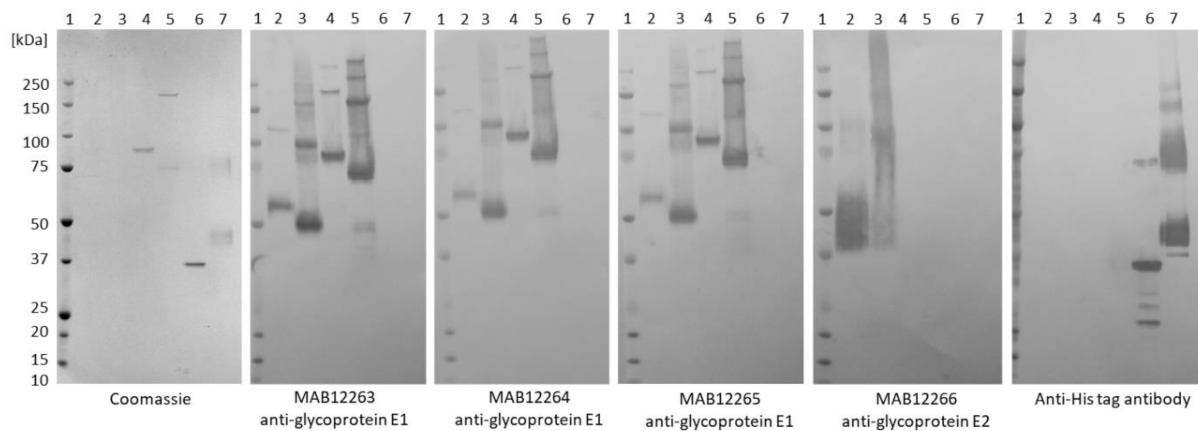

Figure 1. Western blot analysis of different monoclonal antibodies against Rubella VLPs, E1 and NP proteins. Lane 1 shows molecular weight Ladder; lane 2: Rubella VLP (REC31651); lane 3: Rubella VLP (REC31651) non-reducing; lane 4: Rubella E1-shFc (REC31655); lane 5: Rubella E1-shFc (REC31655) non-reducing; lane 6: Rubella NP (REC31668); lane 7: His-tagged control (DENV2 VLP, non-reducing).

2. ELISA

Greiner plates were coated overnight at 4°C with Rubella glycoprotein E1 (REC31655), Rubella VLP (REC31651) or Rubella Nucleoprotein (REC31668) at a concentration of 0.5µg/ml in PBS. Plates were washed once with PBS/0.1% Tween 20 (300µl/well) and blocked twice with PBS 1% BSA (300µl/well) for ~2 h. Antibodies were diluted in PBS/1% BSA/0.1% Tween 20 from 10,000ng/ml to 3ng/ml and added to the plates at 100µl/well and incubated with shaking for 2 h. Plates were then washed three times (300µl/well) with PBS 0.1% Tween 20. Anti-Mouse IgG-HRP was added (1:5000 dilution) in PBS/BSA/Tween (100µl/well) and incubated for 1 h with shaking. Plates were then washed six times in PBS/Tween 20 (300µl/well). KPL Sureblue substrate added (100µl/well), and the plate was incubated with shaking 15 min. and stopped with 200µl 1M HCL.