Zika Virus IgG/IgM/IgA ELISA
Quickstart

PREP

1. Dilute the 25x wash buffer 1 in 25 with purified lab grade water to make 1x wash buffer
2. Reconstitute the Negative and Positive Controls in 500µl of **Sample Diluent**. Reconstitute Differential Control in **Sample Diluent** using the volume indicated on label. Leave for 10 minutes. Vortex before use.
3. Dilute each sample to be analysed and each control 1 in 101 in **Sample Diluent.** Mix well.
4. Reconstitute **Detection Reagent 1** in 3ml of **Reagent 1 diluent**, vortex and allow it to stand for 10 minutes. *Vortex again just before addition to the plate.*

ASSAY *(1hr 45min)*

1. Add 100µl of the diluted controls and samples (in **Sample Diluent**) in duplicate wells, cover with plate sealer and incubate for 30 minutes at room temperature on a shaker set to 800rpm.
2. Aspirate the plate contents, wash 3 times with 300µl of 1x wash buffer and pat dry on absorbent paper.
3. Add 100µl of the **Detection reagent 1** to every well used. Cover, incubate and shake (30 mins, ambient, 800rpm).
4. Repeat wash from step 2 but with 5 washes.
5. Add 100µl of the **Detection reagent 2** to every well used. Cover, incubate and shake (30 mins, ambient, 800rpm).
6. Repeat wash from step 2 but with 5 washes.
7. Add 100µl of TMB substrate to every well used. Cover with new plate sealer and incubate **15 minutes** with shaking 800rpm at ambient temperature.
8. Add 100µl stop solution to every well used. Read with plate reader at 450nm (620nm reference) within **15 minutes** of stopping the reaction.