

## Quick Start Guide: Avidity Rubella Virus IgG ELISA [ELS61245]

Enzyme immunoassay for determination of Rubella-specific IgG avidity. For research use only.

### (A) Preparation of Reagents

Bring all reagents and samples to room temperature (20-25 °C) and mix before starting.

- **Avidity Reagent** - If crystals have formed in the reagent warm up to 37°C and mix gently.
- **Washing Buffer** - Fill 15 ml ready to use Washing Buffer into supplied bottle.

### (B) Assay Steps -For avidity determination dual pipetting of standards/controls and diluted samples is needed.

1. Dispense 100 µl standards/controls and diluted samples into their respective wells. Leave wells A1/A2 for the Substrate Blank. Cover wells with the foil supplied in the kit.
2. **Incubate for 1 hour ± 5 min at 37 ± 1 °C.**
3. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µl of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!
4. Dispense 100 µl of Avidity Reagent in wells B1, C1, D1, E1 etc, except for the Substrate Blank well A1.
5. Dispense 100 µl of Washing Buffer in wells B2, C2, D2, E2 etc, except for the Substrate Blank well A2.
6. **Incubate for exactly 10 min at 37 ± 1 °C.**
7. Repeat step 3.
8. Dispense 100 µl Conjugate into all wells except in the blank wells (A1/A2).
9. **Incubate for 30 min at room temperature (20-25 °C).** Do not expose to direct sunlight.
10. Repeat step 3.
11. Dispense 100 µl TMB Substrate Solution into all wells.
12. **Incubate for exactly 15 min at room temperature (20-25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
13. Dispense 100 µl Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate, thereby a colour change from blue to yellow occurs.
14. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.