Quick Start Guide: Legionella IgG ELISA [ELS61256]

Enzyme immunoassay for the qualitative determination of IgG class antibodies against Legionella pneumophila in human serum or plasma.

(A) Preparation of Reagents

- It is very important to bring all reagents and samples to room temperature (20...25 °C) and mix them thoroughly before starting.
- Dilute Washing Buffer 1 + 19; e. g. 10 ml Washing Buffer + 190 ml distilled water.
- All samples should be diluted 1+100 with IgG Sample Diluent. Dispense 10 µl sample and 1 ml IgG Sample Diluent into tubes to obtain a 1+100 dilution and thoroughly mix.

(B) Assay Steps

1. Dispense 100 µl standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. Incubate for 1 hour ± 5 min at 37 ± 1 °C.
4. 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 und aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!
5. Dispense 100 µl Conjugate into all wells except for the Substrate Blank well A1.
6. Incubate for 30 min at room temperature (20...25 °C). Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µl TMB Substrate Solution into all wells.
9. Incubate for exactly 15 min at room temperature (20...25 °C) in the dark. A blue colour occurs due to an enzymatic reaction.
10. Dispense 100 µl Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.
12. Adjust the ELISA microwell plate reader to zero using the Substrate Blank. Measure the absorbance of all wells at 450 nm and record the absorbance values for each standard/control and sample in the plate layout. Bichromatic measurement using a reference wavelength of 620 nm is recommended.