

Appendix B
Preparation Reagent for ELISA

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Reagent for Mercodia Insulin ELISA

- Calibrator 0,1,2,3,4,5
- Enzyme Conjugate 11X
- Enzyme Conjugate Buffer
- Wash Buffer 21X
- Substrate TMB
- Stop Solution
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Preparation of enzyme conjugate solution

Prepare the needed volume of enzyme conjugate solution by dilution of Enzyme Conjugate 11X, (1+10) in Enzyme Conjugate Buffer

Preparation of wash buffer

Prepare the needed volume of wash buffer by dilution of Wash Buffer 21X in redistilled water (1+20)

Test Procedure

All reagents and samples must be brought to room temperature before use.

Prepare a calibrator curve for each assay run.

1. Prepare enzyme conjugate solution and wash buffer.
 2. Prepare sufficient microplate wells to accommodate Calibrators and samples in duplicate.
 3. Pipette 25 µl each of Calibrators and samples into appropriate wells.
 4. Add 100 µl of enzyme conjugate solution to each well.
 5. Incubate on a plate shaker for 2 hour at room temperature (18–25°C)
 6. Wash plate 6 times with automatic plate washer or Aspirate the reaction volume. Add 350 µl wash buffer to each well. Aspirate completely. Repeat 5 times.
- After final wash, invert and tap the plate firmly against absorbent paper.

7. Add 200 μ l Substrate TMB into each well
8. Incubate for 15 minutes at room temperature (18–25°C)
9. Add 50 μ l Stop Solution to each well.

Place plate on a shaker for approximately 5 seconds to ensure mixing.

10. Read optical density at 450 nm and calculate results. Read within 30 minutes.