Mercodia
Developing diagnostics

Technical Note No: 34-0132

PROTOCOL: 10 µL SAMPLE APPLICATION IN MERCODIA'S ULTRASENSITIVE MOUSE INSULIN ELISA

The sample volume requirement in Mercodia's Ultrasensitive Mouse Insulin ELISA (10-1249-01/10) has been optimized to 25 µL to achieve the most sensitive, specific and robust assay yielding results with the highest accuracy.

A sample volume of 10 µL can be used with a slightly altered protocol. One should be aware that the assay performance characteristics will be affected:

- The decrease in sample volume will affect the OD of the Calibrators.
- The decrease in sample volume does not affect the intra assay variation, however due to the decrease in signal the curve fitting method is crucial. Without optimal curve fitting, one might get increased inter- and total assay variation compared with using 25 µL sample volume (Optimal curve fitting is a third degree polynomial such as cubic spline or 4 P.L.).
- The detection limit when using 10 µL Calibrator and Sample volume is 0.1 µg/L.

SUMMARY PROTOCOL SHEET

Add Calibrators 2-5, Controls and Samples	10 µL
Add enzyme conjugate 1X solution to all wells	100 µL
Incubate	2 hours at 18-25°C on a plate shaker
Wash plate with wash buffer 1X solution	6 times
Add Substrate TMB	200 µL
Incubate	15 minutes
Add Stop Solution	50 µL Shake for 5 seconds to ensure mixing
Measure A 450	Evaluate results

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