

## **Technical Note**

No: 34-0130

## Protocol: 5 $\mu\text{L}$ sample application in Mercodia Rat Insulin ELISA and Mouse Insulin ELISA

The sample volume requirement in Mercodia Rat Insulin ELISA (10-1250-01/10) and Mouse Insulin ELISA (10-1247-01/10) has been optimized to 10  $\mu$ L to achieve the most sensitive, specific and robust assay yielding results with the highest accuracy.

A sample volume 5  $\mu$ L can be used with a slightly altered protocol. One should be aware of that the assay might not be as sensitive as with 10  $\mu$ L sample volume.

Add Calibrators, Controls and Samples	5 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	30 minutes
Add Stop Solution	50 µL
	Shake for 5 seconds to ensure mixing
Measure A <sub>450</sub>	Evaluate results

## Summary protocol sheet