



# Equine insulin immunoassay kit

Catalogue number: 36009

For the quantitative determination of insulin  
in equine serum and plasma samples

This package insert must be read in its entirety before using this product

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## **PRINCIPLE OF THE ASSAY**

This assay is a two-site ELISA. The micro-plate is pre-coated with a monoclonal antibody against insulin. Standards and samples are added into the wells and co-incubated with a monoclonal antibody conjugated to horseradish peroxidase (HRP) enzyme. After wash step to remove any unbound substances, TMB substrate is added and colour develops in proportion to the amount of insulin bound initially. The assay is stopped and the optical density of the wells determined using a micro-plate reader. Since the increases in absorbance are directly proportional to the amount of captured insulin, the unknown sample concentration can be interpolated from a reference curve included in each assay.

## **REAGENTS SUPPLIED**

*Each kit is sufficient for one 96-well plate and contains the following components:*

1. Micro-titre Strips (96 wells)-Coated with a monoclonal antibody against insulin, sealed.
2. (10×) Wash buffer-30 ml.
3. Assay buffer-13 ml, ready for use.
4. (100×) Detection antibody solution-A monoclonal antibody against insulin conjugated to horseradish peroxidase (0.12 ml).
5. Insulin standard solutions-0  $\mu\text{U/ml}$  (5 ml). 3  $\mu\text{U/ml}$ , 6  $\mu\text{U/ml}$ , 12  $\mu\text{U/ml}$ , 25  $\mu\text{U/ml}$ , 50  $\mu\text{U/ml}$ , 120  $\mu\text{U/ml}$  (0.3 ml each), ready for use.
6. Substrate solution- 12 ml, ready for use.
7. Stop solution-12 ml, ready for use.
8. Plate cover-1.

## **OTHER MATERIALS REQUIRED, BUT NOT PROVIDED**

1. Pipettes and pipette tips.
2. 96-well plate or manual strip washer.
3. Buffer and reagent reservoirs.
4. Paper towels or absorbent paper.
5. Plate reader capable of reading absorbency at 450 nm.
6. Distilled water or deionized water.
7. Horizontal micro-plate shaker capable of 600 rpm.

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## STORAGE

The kit should be stored at 2-8°C upon receipt. Remove any unused antibody-coated strips from the micro-plate, return them to the foil pouch and re-seal. Once opened, the strips may be stored at 2-8°C for up to one month.

## PREPARATION OF REAGENTS

*Bring all reagents and materials to room temperature before assay.*

### A. 1×Wash buffer.

Prepare 1×Wash buffer by mixing the 10×Wash buffer (30 ml) with 270 ml of distilled water or deionized water. If precipitates are observed in the 10× Wash buffer bottle, warm the bottle in a 37°C water bath until the precipitates disappear. The 1× Wash buffer may be stored at 2-8°C for up to one month.

### B. 1×Detection antibody solution.

Prepare 1×Detection antibody solution by dilution of the 100×Detection antibody solution in Assay buffer, mix well. 100 µl of the 1×Detection antibody solution is required per well. Prepare only as much 1×Detection antibody solution as needed. Return the 100×Detection antibody solution to 2-8°C immediately after the necessary volume is removed.

## SAMPLE HANDLING

No dilution of the sample is required in this assay, if a sample has an insulin level greater than the highest standard, the sample should be diluted with 0 µU/ml insulin standard solution and the assay should be repeated.

## ASSAY PROCEDURE

*It is recommended that all standards and samples should be run in duplicate.*

1. Add 25 µl of standard or sample to its respective well.
2. Add 100 µl of 1x Detection antibody solution per well.
3. Seal the plate with a plate cover. Incubate at room temperature for 1 hour, shaking the plate at 600 rpm on a horizontal micro-plate shaker.
4. Discard the content and tap the plate on a clean paper towel to remove residual solution in each well. Add 300 µl of 1×Wash buffer to each well. Incubate at room temperature for 20 seconds. Discard the 1×Wash buffer and tap the plate on a clean paper towel to remove residual wash buffer. Repeat the wash step for a total 4 washes.

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5. Add 100  $\mu$ l of Substrate solution to each well, incubate at room temperature for 15 minutes. **Protect from light.**
  6. Add 100  $\mu$ l of Stop solution to each well, gently tap the plate frame for a few seconds to ensure thorough mixing.
  7. Measure absorbance of each well at 450 nm immediately.

### **CALCULATION**

1. Subtract the absorbance of the blank from that of standards and samples.
2. Generate a standard curve by plotting the absorbance obtained (y-axis) against insulin concentrations (x-axis). The best fit line can be generated with any curve-fitting software by regression analysis. Log-log curve fitting or curve of 4-parameter can be used for calculation.
3. Determine insulin concentration of samples from standard curve.
4. Conversion factor: 1  $\mu$ U/ml = 0.0348 ng/ml

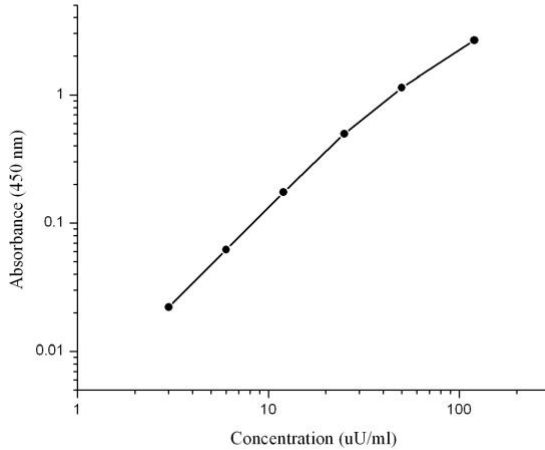
### **TYPICAL STANDARD CURVE**

The following standard curve is provided for demonstration only. A standard curve should be generated for each assay.

Insulin ( $\mu$ U/ml)	Absorbance (450 nm)	Blanked Absorbance
0	0.052	0
3	0.074	0.022
6	0.114	0.062
12	0.226	0.174
25	0.549	0.497
50	1.189	1.137
120	2.705	2.653

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### Insulin standard curve (log-log)



## ASSAY CHARACTERISTICS

### A. Sensitivity

The lowest insulin level that can be measured by this assay is  $3\mu\text{U/ml}$ .

### B. Precision

Intra-assay Precision (Precision within an assay) C.V. < 10%.

Inter-assay Precision (Precision between assays) C.V. < 10%.

### C. Recovery

The recovery of the assay was determined by adding various amounts insulin to a sample. The measured concentration of the spiked sample in the assay was compared to the expected concentration. The average recovery was 92%.

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## SUMMARY OF ASSAY PROCEDURE

