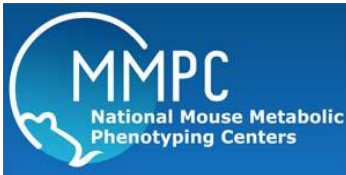

Plasma Insulin Measurement by ELISA

(Case Western Reserve University Test #CA2006)

Summary: Mouse Insulin ELISA kit is used for the non radioactive quantification of insulin in mouse plasma.

Reagents and Materials:

Reagent/Material	Quantity Required	Vendor	Stock Number
Mouse Sensitive Insulin ELISA	10ul serum/plasma per well	Mercodia	nr 10-1247-01
Mouse Ultrasensitive Insulin ELISA	25ul Serum/plasma per well	Mercodia	10-1249-01

Protocol:

1. Plasma or serum are isolated from mice fasted or after glucose injection. Heparin or EDTA coated tubes are acceptable for plasma fractionation.

2. Insulin is measured according to the manufacturer's directions.

Manufacturer's Protocol below:
MOUSE INSULIN ELISA KIT

96-Well Plate (Cat. #10-1247-01)

Principle of procedure (provided by manufacturer)

Mercodia Mouse Insulin ELISA is a solid phase two-site enzyme immunoassay. It is based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the insulin molecule. During incubation insulin in the

sample reacts with peroxidase-conjugated anti-insulin antibodies and anti-insulin antibodies bound to microplate wells. A simple washing step removes unbound enzyme labeled antibody. The bound conjugate is detected by reaction with 3,3’,5,5’-tetramethylbenzidine. The reaction is stopped by adding acid to give a colorimetric endpoint that is read spectrophotometrically. This assay is a Sandwich ELISA based, sequentially, on: 1) capture of insulin molecules from samples to the wells of a microtiter plate coated by pre-titered amount of a monoclonal mouse anti-rat insulin antibodies and the binding of biotinylated polyclonal antibodies to the captured insulin, 2) wash away of unbound materials from samples, 3) binding of horseradish peroxidase to the immobilized biotinylated antibodies, 4) wash away of free enzyme conjugates, and 5) quantification of immobilized antibody-enzyme conjugates by monitoring horseradish peroxidase activities in the presence of the substrate 3,3',5,5'-tetramethylbenzidine. The enzyme activity is measured spectrophotometrically by the increased absorbency at 450 nm, corrected from the absorbency at 590nm, after acidification of formed products. Since the increase in absorbency is directly proportional to the amount of captured insulin in the unknown sample, the latter can be derived by interpolation from a reference curve generated in the same assay with reference standards of known concentrations of rat insulin.