Mercodia
Developing diagnostics

Mercodia Rat/Mouse Insulin FIA

Directions for Use

10-1248-10
REAGENTS FOR 10 x 96 DETERMINATIONS

For Research Use Only

Manufactured by
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EXPLANATION OF SYMBOLS USED ON LABELS

	Reagents for 96 determinations
	Expiry date
	Store between 2–8°C
	Lot No.

INTENDED USE

Mercodia Rat/Mouse Insulin FIA provides a method for the quantitative determination of insulin in rat and mouse serum, plasma or culture medium.

PRINCIPLE OF THE PROCEDURE

Mercodia Rat/Mouse Insulin FIA 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 react with peroxidase-conjugated anti-insulin antibodies and anti-insulin antibodies bound to the microplate well. A simple washing step removes unbound enzyme labelled antibody. The bound conjugate is detected by reaction with a fluorescent substrate. The reaction is stopped by adding stop solution, after which the plate is analyzed for fluorescence with Excitation/Emission 325nm/420nm.

WARNINGS AND PRECAUTION

- For research use only.
- Not for internal or external use in humans or animals.
- The content of this kit and their residues must not be allowed to come into contact with ruminating animals or swine.
- All samples should be handled as if capable of transmitting infections.
- Each well can only be used once.

MATERIAL REQUIRED BUT NOT PROVIDED

- Pipettes with appropriate volumes (repeating pipettes preferred for addition of
- enzyme conjugate 1X solution, Substrate Solution and Stop Solution)
- Tubes, beakers and cylinders for reagent preparation
- Redistilled water
- Magnetic stirrer
- Vortex mixer
- Fluorescence reader (Ex/Em 325/420nm)
- Microplate shaker (700–900 cycles per minute, orbital movement)
- Microplate washing device with overflow function (recommended but not required)

REAGENTS

Each Mercodia Rat/Mouse Insulin FIA kit (10-1248-10) contains reagents for 10x96 wells, each plate sufficient for 40 samples and one calibrator curve in duplicate. For larger series of assays, use pooled reagents from packages bearing identical lot numbers. The expiry date for the complete kit is stated on the outer label. The recommended storage temperature is 2-8°C.

Coated Plate Mouse monoclonal anti-insulin For unused microplate wells, reseal the bag using adhesive tape, store at 2–8°C and use within 8 weeks.	10 plates	96 wells 8-well strips	Ready for Use
Calibrators 1, 2, 3, 4, 5, 6, 7 Rat insulin Color coded yellow Concentration stated on vial label.	7 vials	1000 µL	Ready for Use
Calibrator 0 Color coded yellow	1 vial	30 mL	Ready for Use
Enzyme Conjugate 11X Peroxidase conjugated mouse monoclonal anti-insulin	1 vial	12 mL	Preparation, see below
Enzyme Conjugate Buffer Color coded blue	1 bottle	120 mL	Ready for use
Wash Buffer 21X Storage after dilution: 2-8°C for 8 weeks.	2 bottles	200 mL	Preparation, see below
Substrate Solution Colorless solution Delivered in separate box	1 bottle	250 mL	Preparation. see below
Stable Peroxide Solution Colorless solution Delivered in separate box	1 vial	30 mL	Preparation, see below
Stop Solution Colorless solution Glycine Delivered in separate box	1 bottle	275 mL	Ready for Use

Preparation of enzyme conjugate 1X solution

Prepare the needed volume of enzyme conjugate 1X solution by dilution of Enzyme Conjugate 11X, (1+10) in Enzyme Conjugate Buffer according to the table below. Mix gently.

Number of plates	Enzyme Conjugate 11X	Enzyme Conjugate Buffer
10 plates	1 vial	1 bottle
5 plates	6.0 mL	60 mL
3 plates	3.6 mL	36 mL
2 plates	2.4 mL	24 mL
1 plate	1.2 mL	12 mL

Storage after dilution: 2-8°C for 8 weeks.

Preparation of substrate solution

Prepare the needed volume of substrate solution by mixing Stable Peroxide Solution (1+9) in Substrate Solution according to the table below. Mix gently.

Number of plates	Stable Peroxide Solution	Substrate Solution
20 plates	27 mL	243 mL
10 plates	13 mL	117 mL
5 plates	6.0 mL	54.0 mL
3 plates	4.0 mL	36.0 mL
2 plates	2.5 mL	22.5 mL
1 plate	1.3 mL	11.7 mL

Storage after dilution: 2-8°C for 1 day.

Preparation of wash buffer 1X solution

Prepare the needed volume of wash buffer 1X solution by dilution of Wash Buffer 21X in redistilled water 1+20 according to the table below. Mix gently.

Number of plates	Wash buffer 21X	Redistilled water
10 plates	2 bottles	8000 mL
5 plates	180 mL	3600 mL
3 plates	110 mL	2200 mL
2 plates	70 mL	1400 mL
1 plate	35 mL	700 mL

Storage after dilution: 2-8°C for 8 weeks.

SPECIMEN COLLECTION AND HANDLING

Serum

Collect blood by venipuncture, allow to clot, and separate the serum by centrifugation. Specimen may be stored for 24 hours at 2-8°C. For longer periods store samples at -20°C. Avoid repeated freezing and thawing.

Plasma

Collect blood by venipuncture into tubes containing EDTA or heparin as anticoagulant, and separate the plasma fraction by centrifugation. Specimen may be stored for 24 hours at 2-8°C. For longer periods store samples at -20°C. Avoid repeated freezing and thawing.

PREPARATION OF SAMPLES

No dilution is normally required, however, samples containing >100µg/L should be diluted 1/10 v/v with Calibrator 0. *Note!* Buffers containing sodium azide (NaN_3) cannot be used for sample dilution.

TEST PROCEDURE

All reagents and samples must be brought to room temperature before use. Prepare a calibrator curve for each assay run. Avoid pipetting solution onto the walls.

1. Prepare enzyme conjugate 1X solution, wash buffer 1X solution and substrate solution.
2. Prepare sufficient microplate wells to accommodate Calibrators, controls and samples in duplicate.
3. Pipette 10 µL each of Calibrators, controls and samples into appropriate wells.
4. Add 100 µL of enzyme conjugate 1X solution into each well.
5. Incubate on a plate shaker (700-900 rpm) for 2 hours at room temperature (18-25°C).
6. Wash 6 times with 700 µL wash buffer 1X solution per well using an automatic plate washer with overflow-wash function. After final wash, invert and tap the plate firmly against absorbent paper. Do not include soak step in washing procedure.
   Or manually,
   discard the reaction volume by inverting the microplate over a sink. Add 350 µL wash buffer 1X solution to each well. Discard the wash solution, tap firmly several times against absorbent paper to remove excess liquid. Repeat 5 times. Avoid prolonged soaking during washing procedure.
7. Add 100 µL substrate solution into each well.
8. Incubate on the bench for 15 minutes at room temperature (18-25°C).
9. Add 100 µL Stop Solution to each well.
   Place the plate on the shaker for approximately 5 seconds to ensure mixing.
10. Read fluorescence at Ex/Em 325nm/420nm with optimal gain according to instructions from fluorescent reader supplier.
    Read within 30 minutes.

Note! To prevent contamination between the conjugate and substrate, separate pipettes are recommended.

INTERNAL QUALITY CONTROL

Commercial controls and/or internal serum pools with low, intermediate and high rat or mouse insulin concentrations should routinely be assayed as samples, and results charted from day to day. It is good laboratory practice to record the following data for each assay: kit lot number, dilution dates of kit components, RFU values for the blank, Calibrators and controls.

Laboratories should follow government regulations or accreditation requirements for quality control frequency.

CALCULATIONS OF RESULTS

Computerized calculations

The concentration of insulin is obtained by computerized data reduction of the fluorescence for the Calibrators, except Calibrator 0, versus the concentration using cubic spline regression.

Manual calculation

1. Plot the fluorescence values obtained for the Calibrators, except Calibrator 0, against the insulin concentration on a log-log paper and construct a calibrator curve.
2. Read the concentration of the unknown samples from the calibrator curve.
3. If the sample is diluted: Multiply the concentration with the dilution factor.

Example of results

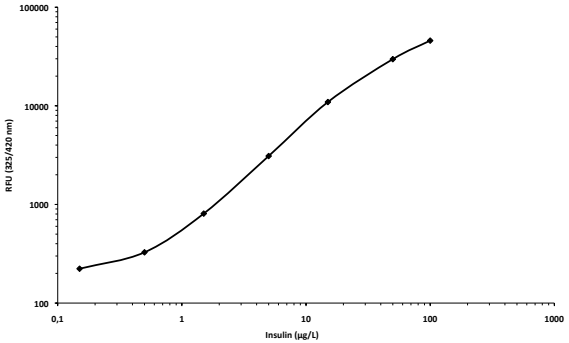
These values were obtained using Tecan's Infinite M200 with 63 gain, 25 number of flashes, 0 ms settle time, 0 µs lag time and 20 µs intergration time.

Wells	Identity	RFU $325/420_{Ex/Em}$	Mean conc. µg/L
1 A-B	Calibrator 0	197/189	
2 C-D	Calibrator 1*	224/222	
3 E-F	Calibrator 2*	331/323	
4 G-H	Calibrator 3*	798/817	
2 A-B	Calibrator 4*	3153/3039	
2 C-D	Calibrator 5*	10911/10947	
2 E-F	Calibrator 6*	29508/29948	
2 G-H	Calibrator 7*	46033/4566	
3 A-B	Sample 1	440/443	0.766
3 C-D	Sample 2	2589/2526	4.257
3 E-F	Sample 3	16200/16162	22.699

* Concentration stated on vial label.

Example of calibrator curve

A typical calibrator curve is shown below. Do not use this curve to determine actual assay results.



Conversion factor

1 µg corresponds to 174 pmol.

LIMITATIONS OF THE PROCEDURE

Performance limitations

Grossly lipemic, icteric or haemolyzed samples do not interfere in the assay. Insulin is, however, degraded over time in heamolyzed samples. The degradation could give falsely low values and contributes to higher inter-assay variation.

Separate pipettes should be used when pipetting the conjugate and the substrate.

EXPECTED VALUES

Good practice dictates that each laboratory establishes its own expected range of values.

PERFORMANCE CHARACTERISTICS

Detection limit

Detection limit is defined as the Capability of Detection according to ISO11843-Part 1. Capability of Detection should be seen as a part of a method validation, rather than the lowest concentration that can be measured.

The detection limit is ≤0.15 µg/L as determined with the methodology described in ISO11843-Part 4.

Concentration of samples with fluorescence below Calibrator 1 should not be calculated, instead expressed less or equal to (≤) the concentration indicated on the vial for Calibrator 1.

Recovery

Recovery upon addition is 88-124 % (mean 101 %).
Recovery upon dilution is 98-121 % (mean 108 %).

Hook effect

Samples with an insulin concentration up to at least 2 800 µg/L can be measured without giving falsely low results.

Precision

Each sample was analyzed in 4 replicates on 30 different occasions.

Sample	Mean value µgl/L	Coefficient of variation		
		within assay %	between assay %	total assay %
1	0.96	3.3	4.6	4.9
2	4.68	3.4	4.1	4.5
3	26.1	2.9	6.8	7.0

Specificity

Mouse C-peptide I	<0.002%
Mouse C-peptide II	<0.001%
Mouse Proinsulin I	33%
Mouse Proinsulin II	51%
Rat C-peptide I	<0.03%
Rat C-peptide II	<0.03%
Rat Proinsulin I	8%
Rat Proinsulin II	51%
Human Insulin	167%
Human C-peptide	< 0.05%
Human Proinsulin	75%
Insulin lispro (Humalog®)	167%
IGF-I	< 0.02%
IGF-II	< 0.02%
Porcine Insulin	476%
Ovine Insulin	179%
Bovine Insulin	78%

CALIBRATION

Mercodia Rat/Mouse Insulin FIA is calibrated against an in-house reference preparation of recombinant rat insulin I.

WARRANTY

The performance data presented here was obtained using the procedure indicated. Any change or modification in the procedure not recommended by Mercodia AB may affect the results, in which event Mercodia AB disclaims all warranties expressed, implied or statutory, including the implied warranty of merchantability and fitness for use.

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SUMMARY OF PROTOCOL SHEET

Mercodia Rat/Mouse Insulin FIA

Add Calibrators, controls* and samples	10 µL
Add enzyme conjugate 1X solution	100 µL
Incubate	2 hour at 18-25°C on a plate shaker 700-900 rpm
Wash plate with wash buffer 1X solution	700 µL, 6 times
Add substrate solution	100 µL
Incubate	15 minutes at 18-25°C
Add Stop Solution	100 µL Shake for 5 seconds to ensure mixing
Measure $325/420_{Ex/Em}$	Evaluate results

*not provided

For full details see page 6