

Merckodia

Rat Insulin ELISA

Enzyme immunoassay

Directions for Use

10-1124-01

REAGENTS FOR 96 DETERMINATIONS

10-1124-10

REAGENTS FOR 10 × 96 DETERMINATIONS

For Research Use Only





Not for Use in Diagnostic Procedures

Manufactured by

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EXPLANATION OF SYMBOLS USED ON LABELS

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

INTENDED USE

Mercodia Rat Insulin ELISA provides a method for the quantitative determination of insulin in rat serum or plasma.

PRINCIPLE OF THE PROCEDURE

Mercodia Rat 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 react with peroxidase-conjugated anti-insulin antibodies and anti-insulin antibodies bound to microtitration well. A simple washing step removes unbound enzyme labelled 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.

WARNINGS AND PRECAUTIONS

- For reserach use only. Not for use in diagnostic procedures.
- 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.
- The Stop Solution in this kit contains 0.5 M H₂SO₄. Follow routine precautions for handling hazardous chemicals.

SPECIMEN COLLECTION AND HANDLING

Serum

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

Plasma

Collect blood by venipuncture into tubes containing heparin or EDTA as anticoagulant, and separate the plasma fraction. Samples can be stored at 2–8°C up to 24 hours. For longer periods store samples at –20°C. Avoid repeated freezing and thawing.

Preperation of samples

No dilution is normally required, however, samples containing >5.5 µg/l should be diluted 1/10 v/v with Calibrator 0. Note! Buffers containing sodium azide (NaN₃) can not be used for sample dilution.

MATERIAL REQUIRED BUT NOT PROVIDED

- 25 µl micropipette with disposable tips
- EIA plate reader with 450 nm filter
- Wash device for microtitration plates
- 50 µl and 200 µl repeating pipettes
- Tube (10-100 ml) for preparation of Conjugate
- Redistilled water
- 1000 ml /10 l bottle
- Plate shaker

(The recommended velocity is 700-900 cycles per minute, orbital movement)

REAGENTS 1 X 96 KIT

Each Mercodia Rat Insulin ELISA kit (10-1124-01) contains reagents for 96 wells, sufficient for 42 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)	1 plate 8-well strips	96 wells	Ready for use
For unused microplate wells, completely reseal the bag using adhesive tape and use within two months.			
Calibrators (Rat Insulin) 0.15; 0.4; 1.0; 3.0 and 5.5 µg/l	5 vials	1000 µl	Ready for use
Calibrator 0 Color coded yellow	1 vial	5 ml	Ready for use
Enzyme Conjugate 11X (Peroxidase conjugated mouse monoclonal anti-insulin)	1 vial	600 µl	Preparation, see below
Enzyme Conjugate Buffer Color coded blue	1 vial	6 ml	Ready for use
Wash Buffer 21X	1 bottle	40 ml	Dilute 1+20 with 800 ml redist. water to make Wash Buffer
Substrate TMB <i>Note! Light sensitive!</i>	1 vial	22 ml	Ready for use
Stop Solution 0.5 M H ₂ SO ₄	1 vial	7 ml	Ready for use

Preparation of enzyme conjugate solution

Prepare the needed volume of enzyme conjugate solution by mixing 50 μ l Enzyme Conjugate 11X with 500 μ l Enzyme Conjugate buffer (1+10) for each strip or as in the table below.

When preparing enzyme conjugate solution for the whole plate, pour all of the Enzyme Conjugate Buffer into the Enzyme Conjugate 11X vial.

Number of strips	Enzyme Conjugate 11X	Enzyme Conjugate buffer
12 strips	1 vial	1 vial
6 strips	300 μ l	3 ml
4 strips	200 μ l	2 ml

Storage after dilution: 2–8°C for two months.

REAGENTS 10 X 96 KIT

Each Mercodia Rat Insulin ELISA kit (10-1124-10) contains reagents for 10 × 96 wells, sufficient for 42 samples and one Calibrator curve in duplicate on each plate. 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)	10 plates 8-well strips	à 96 wells	Ready for use
For unused microplate wells, completely reseal the bag using adhesive tape and use within two months.			
Calibrators 0.15; 0.4; 1.0; 3.0 and 5.5 µg/l	5 vials	1000 µl	Ready for use
Calibrator 0 Color coded yellow	1 vial	5 ml	Ready for use
Enzyme Conjugate 11X (Peroxidase conjugated mouse monoclonal anti-insulin)	1 vial	6 ml	Preparation, see below
Enzyme Conjugate buffer Color coded blue	1 bottle	60 ml	Ready for use
Wash Buffer 21X	1 bottle	400 ml	Preparation, see below
Substrate TMB (TMB) Colorless solution <i>Note! Light sensitive!</i>	1 bottle	220 ml	Ready for use
Stop Solution 0.5M H ₂ SO ₄	1 bottle	70 ml	Ready for use

Preparation of enzyme conjugate solution

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

Number of plates/ strips	Enzyme Conjugate 11X	Enzyme Conjugate buffer
10 plates	1 vial	1 bottle
5 plates	3000 μ l	30 ml
3 plates	1800 μ l	18 ml
2 plates	1200 μ l	12 ml
1 plate	600 μ l	6 ml

Storage after dilution: 2–8°C for two months.

Preparation of Wash Buffer

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

Number of plates/ strips	Wash Buffer 21X	Redistilled water
10 plates	1 bottle	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 four weeks.

TEST PROCEDURE

Perform each determination in duplicate for Calibrators and unknowns. Prepare a Calibration curve for each assay run. All reagents and samples must be brought to room temperature before use.

Add to anti-Insulin wells	Calibrators	Unknowns
1 Calibrators	25 μ l	–
2 Unknowns	–	25 μ l
3 Enzyme conjugate solution	50 μ l	50 μ l
4 Incubate on a shaker for 2 hours at room temperature.		
5 Wash 6 times with automatic washer or Aspirate the reaction volume. Add 350 μ l Wash Buffer to each well. Aspirate completely. Repeat 5 times. After final wash, invert and tap the plate firmly against absorbent paper.		
6 Substrate TMB	200 μ l	200 μ l
7 Incubate for 15 minutes		
8 Stop Solution	50 μ l	50 μ l
Put the plate on the shaker for approximately 5 seconds to ensure mixing of Substrate and Stop Solution.		
9 Measure the absorbance at 450 nm and evaluate.		

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

INTERNAL QUALITY CONTROL

Commercial controls such as Mercodia Insulin Control Mammalian (Code No. 10-1135-01) and/or internal serum pools with low, intermediate and high insulin concentrations should routinely be assayed as unknowns, and results charted from day to day. It is good laboratory practice to record the following data for each assay: kit lot number; reconstitution dates of kit components; OD values for the blank, Calibrators and controls.

CALCULATION OF RESULTS

Computerized calculation

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

Manual calculation

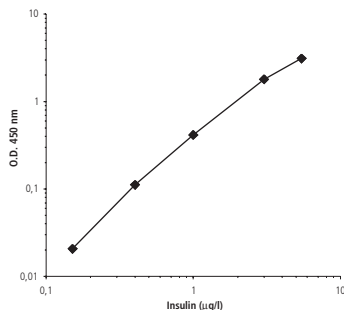
1. Plot the absorbance values obtained for the Calibrators, except Calibrator 0, against the insulin concentration on a log-log or lin-log paper and construct a Calibration curve.
2. Read the concentration of the unknown samples from the Calibration curve.

Example of results

Mean Wells	Identity	A ₄₅₀	conc. µg/l
1A–B	Calibrator 0	0.070/0.067	
1C–D	Calibrator 0.15 µg/l	0.084/0.088	
1E–F	Calibrator 0.4 µg/l	0.141/0.143	
1G–H	Calibrator 1.0 µg/l	0.398/0.400	
2A–B	Calibrator 3.0 µg/l	1.318/1.338	
2C–D	Calibrator 5.5 µg/l	2.337/2.443	
2E–F	Unknown 1	0.760/0.802	1.79
2G–H	Unknown 2	1.032/1.039	2.35
3A–B	Unknown 3	1.878/1.889	4.29

Calibration curve

A typical Calibration curve is shown here. 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 hemolyzed samples do not interfere in the assay.

EXPECTED VALUES

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

PERFORMANCE CHARACTERISTICS

Detection limit

The detection limit is 0.07 µg/l calculated as two standard deviations above the zero Calibrator.

Recovery

Recovery upon addition is 97%

Hook effect

Samples with a concentration of up to at least 576 µg/l can be measured without giving falsely low results.

Precision

Each sample was analysed in 4-replicates on eight different occasions.

Sample	Mean value µg/l	Coefficient of variation		
		within assay %	between assay %	total assay %
1	0.613	3.3	2.0	3.9
2	1.181	3.4	1.2	3.6
3	3.321	3.1	2.2	3.8

SPECIFICITY

Human insulin	167%
Human proinsulin	75%
Human C-peptide	< 0.05%
Insulin lispro (Humalog®)	167%
IGF-I	< 0.02%
IGF-II	< 0.02%
Rat C-peptide	< 0.001%
Rat proinsulin	7%
Mouse insulin	75%
Porcine Insulin	476%
Sheep Insulin	179%
Bovine Insulin	78%

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. Mercodia AB and its authorized distributors, in such event, shall not be liable for damages indirect or consequential.

REFERENCES

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Olsson R, Carlsson PO: Better vascular engraftment and function in pancreatic islets transplanted without prior culture. *Diabetologia* 48:469-476, 2005

Rydtren T, Sandler S: Efficacy of 1400 W, a novel inhibitor of inducible nitric oxide synthase, in preventing interleukin-1 β -induced suppression of pancreatic islet function in vitro and multiple low-dose streptozotocin-induced diabetes in vivo. *Eur J Endocrinol* 147:543-551, 2002

SUMMARY PROTOCOL SHEET
Mercodia Rat Insulin ELISA

Add Calibrators and samples	25 μ l
Add enzyme conjugate solution	50 μ l
Incubate	2 hours at 18–25°C on a shaker
Wash	6 times
Add Substrate TMB	200 μ l
Incubate	15 minutes
Add Stop Solution	50 μ l Shake for 5 seconds to ensure mixing
Measure A_{450}	