

Mercodia Ultrasensitive Mouse Insulin ELISA

Enzyme immunoassay

Directions for Use

10-1150-01

REAGENTS FOR 96 DETERMINATIONS

10-1150-10

REAGENTS FOR 10 X 96 DETERMINATIONS

Note! Calibrator concentrations are indicated on vial labels!

For Research Use Only





Not for Use in Diagnostic Procedures

Manufactured by

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Mercodia 

EXPLANATION OF SYMBOLS USED ON LABELS

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

INTENDED USE

Mercodia Ultrasensitive Mouse Insulin ELISA provides a method for the quantitative determination of insulin in mouse serum or plasma.

PRINCIPLE OF THE PROCEDURE

Mercodia Ultrasensitive 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 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 research 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 0.5M 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 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.

Preparation of samples

No dilution is normally required, however, samples containing insulin concentration above the highest Calibrator should be diluted 1/10 v/v with Calibrator 0.

Note! Buffers containing sodium azid (NaN₃) can not be used for sample dilution.

MATERIAL REQUIRED BUT NOT PROVIDED

- 5 μ l, 25 μ l or 50 μ l micropipette with disposable tips
- 50 μ l and 200 μ l repeating pipettes
- EIA plate reader with 450 nm filter
- Wash device for microtitration plates
- Tube (10–100ml) for preparation of Conjugate
- 1000 ml/10 l bottle
- Redistilled water
- Plate shaker (The recommended velocity is 700-900 cycles per minut, orbital movement)

REAGENTS FOR 1 X 96 KIT

Each Mercodia Ultrasensitive Mouse Insulin ELISA kit (10-1150-01) 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	7 vials	1000 µl	Lyophilized Add 1000 µl redistilled water
Concentration indicated on vial label. Use within 1 week. For storage longer than 1 week, store at –20°C.			
Calibrator 0	1 vial	5 ml	Ready for use
Color coded yellow			
Enzyme Conjugate 11X	1 vial	600 µl	Preparation, see below
(Peroxidase conjugated mouse monoclonal anti-insulin)			
Enzyme Conjugate Buffer	1 vial	6 ml	Ready for use
Color coded blue			
Wash Buffer 21X	1 bottle	40 ml	Dilute 1+20 with 800 ml redistilled water to make Wash Buffer
Substrate TMB	1 vial	22 ml	Ready for use
<i>Note! Light sensitive!</i>			
Stop Solution	1 vial	7 ml	Ready for use
0.5 M H ₂ SO ₄			

Preparation of enzyme conjugate solution

Prepare the needed volume of enzyme conjugate solution by mixing 50 μl Enzyme Conjugate 11X with 500 μl Enzyme Conjugate Buffer (1+10) for each strip or as indicated 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. Mix gently.

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

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

REAGENTS FOR 10 X 96 KIT

Each Mercodia Ultrasensitive Mouse Insulin ELISA kit (10-1150-10) contains reagents for 10 x 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	96 wells	Ready for use
For unused microplate wells, completely reseal the bag using adhesive tape and use within two months.			
Calibrators	7 vials	1000 µl	Lyophilized Add 1000 µl redistilled water
Concentration indicated on vial label. Use within 1 week. For storage longer than 1 week, store at –20°C.			
Calibrator 0	1 vial	30 ml	Ready for use
Color coded yellow			
Enzyme Conjugate 11X	1 vial	6 ml	Preparation, see below
(Peroxidase conjugated mouse monoclonal anti-insulin)			
Enzyme Conjugate Buffer	1 vial	60 ml	Ready for use
Color coded blue			
Wash Buffer 21X	1 bottle	400 ml	Dilute 1+20 with 8000 ml redistilled water to make Wash Buffer
Substrate TMB	1 vial	220 ml	Ready for use
<i>Note! Light sensitive!</i>			
Stop Solution	1 vial	70 ml	Ready for use
0.5 M H ₂ SO ₄			

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 FOR 5 μ L SAMPLE VOLUME

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.

Note! 25 μ l Calibrator 0 must be added to each well before addition of unknowns/Calibrators. Use Calibrator 0 and 3-7.

Add to anti-insulin wells	Calibrators	Unknowns
1 Calibrator 0	25 μ l	25 μ l
2 Calibrators 0, 3-7	5 μ l	–
3 Unknowns	–	5 μ l
4 Enzyme conjugate solution	50 μ l	50 μ l
5 Incubate on a shaker for 2 hours at room temperature (18–25°C).		
6 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.		
7 Substrate TMB	200 μ l	200 μ l
8 Incubate for 30 minutes		
9 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.		
10 Measure the absorbance at 450 nm and evaluate.		

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

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 for 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.

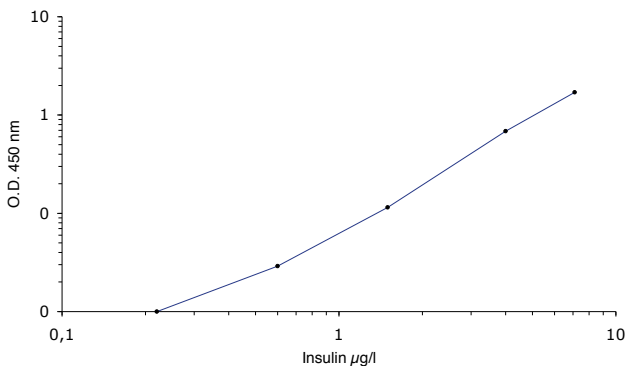
Example of results for 5 µl sample volume

Wells	Identity	Mean A_{450}	Mean conc. µg/l
1A-B	Calibrator 0	0.077	
1C-D	Calibrator 3 (0.22 µg/l*)	0.087	
1E-F	Calibrator 4 (0.60 µg/l*)	0.106	
1G-H	Calibrator 5 (1.5 µg/l*)	0.192	
2A-B	Calibrator 6 (4.0 µg/l*)	0.762	
2C-D	Calibrator 7 (7.1 µg/l*)	1.781	
2E-F	Unknown 1	0.124	0.86
2G-H	Unknown 2	0.229	1.75
3A-B	Unknown 3	0.781	4.06

* Concentration indicated on vial label.

Calibration curve for 5 µl sample volume

A typical Calibration curve is shown here. Do not use this curve to determine actual assay results.



TEST PROCEDURE FOR 50 µL SAMPLE VOLUME

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.

Use Calibrators 0 and 1-5.

Add to anti-insulin wells	Calibrators	Unknowns
1 Calibrators 0, 1-5	50 µl	–
2 Unknowns	–	50 µl
3 Enzyme conjugate solution	50 µl	50 µl
4 Incubate on a shaker for 2 hours at room temperature (18–25°C).		
5 Wash 6 times with automatic washer or Aspirate the reaction volume. Add 350 µl 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 µl	200 µl
7 Incubate for 30 minutes		
8 Stop Solution	50 µl	50 µl
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.

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 for 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.

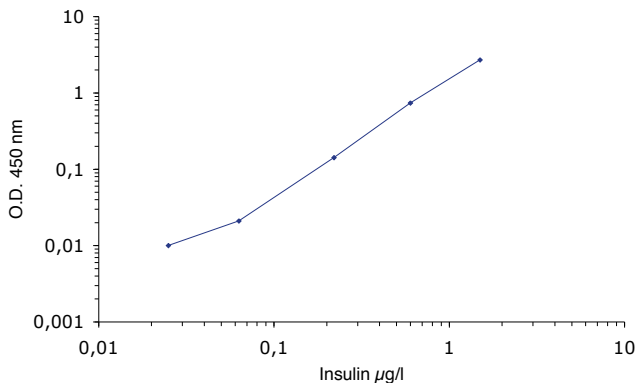
Example of results for 50 µl sample volume

Wells	Identity	Mean A_{450}	Mean conc. µg/l
1A–B	Calibrator 0	0.065	
1C–D	Calibrator 1 (0.025 µg/l*)	0.075	
1E–F	Calibrator 2 (0.063 µg/l*)	0.086	
1G–H	Calibrator 3 (0.22 µg/l*)	0.207	
2A–B	Calibrator 4 (0.60 µg/l*)	0.803	
2C–D	Calibrator 5 (1.5 µg/l*)	2.776	
2E–F	Unknown 1	0.170	0.18
2G–H	Unknown 2	0.393	0.36
3A–B	Unknown 3	1.762	1.06

* Concentration indicated on vial label.

Calibration curve for 50 µl sample volume

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.

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.

LIMITATIONS OF PROCEDURE

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 calculated as two standard deviations above the Calibrator 0.

5 μ l sample: $\leq 0.188 \mu\text{g/l}$

50 μ l sample: $\leq 0.025 \mu\text{g/l}$

Recovery

5 μ l sample: Recovery upon addition is 99%.

50 μ l sample: Recovery upon addition is 95%.

Hook effect

Samples up to at least 346 $\mu\text{g/l}$ can be measured without giving falsely low results.

Precision

5 μ l: Each sample was analysed in 4-replicates on seventeen different occasions.

Sample	Mean value $\mu\text{g/l}$	Coefficient of variation		
		within assay %	between assay %	total assay %
1	0.82	10.2	9.4	13.9
2	1.55	9.8	8.9	13.2
3	4.10	6.2	5.1	8.0

50 µl: Each sample was analysed in 4-replicates on eighteen different occasions.

Sample	Mean value µg/l	Coefficient of variation		
		within assay %	between assay %	total assay %
1	0.82	3.9	7.8	8.7
4	0.42	2.4	8.6	8.9
5	0.25	5.7	8.0	9.8

SPECIFICITY

Human insulin	195%
Human proinsulin	82%
Human C-peptide	< 0.05%
IGF-I	< 0.02%
IGF-II	< 0.02%
Rat insulin	146%
Rat proinsulin	14%
Rat C-peptide	< 0.001%
Porcine insulin	628%
Sheep insulin	256%
Bovine insulin	110%

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

Blyszczuk P, Czyz J, Kania G, Wagner M, Roll U, St-Onge L, Wobus AM: Expression of Pax4 in embryonic stem cells promotes differentiation of nestin-positive progenitor and insulin-producing cells. *Proc Natl Acad Sci U S A* 100:998-1003, 2003

Burcelin R, Crivelli V, Perrin C, Da Costa A, Mu J, Kahn BB, Birnbaum MJ, Kahn CR, Vollenweider P, Thorens B: GLUT4, AMP kinase, but not the insulin receptor, are required for hepatportal glucose sensor-stimulated muscle glucose utilization. *J Clin Invest* 111:1555-1562, 2003

Friesen NT, Buchau AS, Schott-Ohly P, Lgssiar A, Gleichmann H: Generation of hydrogen peroxide and failure of antioxidative responses in pancreatic islets of male C57BL/6 mice are associated with diabetes induced by multiple low doses of streptozotocin. *Diabetologia* 47:676-685, 2004

Jaeckel E, Lipes MA, von Boehmer H: Recessive tolerance to preproinsulin 2 reduces but does not abolish type 1 diabetes. *Nat Immunol* 5:1028-1035, 2004

SUMMARY PROTOCOL SHEET
Merckodia Ultrasensitive Mouse Insulin ELISA

X-O Graf Tryckent AB

Calibrators	0 and 3-7	0 and 1-5
Add Calibrator 0	25 µl	
Add Calibrators and samples	5 µl	50 µl
Add enzyme conjugate solution	50 µl	50 µl
Incubate	2 hours at 18–25°C on a shaker	
Wash	6 times	
Add Substrate	200 µl	
Incubate	30 minutes	
Add Stop Solution	50 µl <i>Shake for 5 seconds to ensure mixing</i>	
Measure A ₄₅₀		