

# Mercodia

# Mouse Insulin ELISA

Enzyme immunoassay

Directions for Use

**10-1149-01**

**REAGENTS FOR 96 DETERMINATIONS**

**10-1149-10**

**REAGENTS FOR 10 X 96 DETERMINATIONS**





**Note!** Calibrator concentrations are indicated on vial labels!

Not for Human Use

Manufactured by  
Mercodia AB, Sylveniusgatan 8A,  
SE-754 50 Uppsala, Sweden

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

## PRINCIPLE OF THE PROCEDURE

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 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

- Not for Human Use.
- 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 H<sub>2</sub>SO<sub>4</sub>. 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 >5.5 µg/l should be diluted 1/10 v/v with Calibrator 0 µg/l. Note! Buffers containing sodium azide (NaN<sub>3</sub>) can not be used for sample dilution.

## MATERIAL REQUIRED BUT NOT PROVIDED

- 25 µl and 1000 µl micropipette with disposable tips
- 50 µl and 200 µl 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

## REAGENTS FOR 1 X 96 KIT

Each Mercodia Mouse Insulin ELISA kit (10-1149-01) 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.

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### Coated Plate

(mouse monoclonal anti-insulin)

1 plate

96 wells

Ready for use

8-wells strips

For unused micro plate wells, completely reseal the bag using adhesive tape and use within two months.

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### Calibrators

Concentration indicated on vial label.

5 vials

1000 µl

Lyophilized

Add 1.0 ml redistilled water.

Use within 1 week. For storage longer than 1 week, store at –20°C.

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### Calibrator 0

Colour coded yellow

1 vial

5 ml

Ready for use

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### Enzyme Conjugate 11X

(Peroxidase conjugated mouse monoclonal anti-insulin, 4.4 µg/l)

1 vial

600 µl

Preparation, see below

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### Enzyme Conjugate Buffer

Colour coded blue

1 vial

6 ml

Ready for use

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### Wash Buffer 21X

1 bottle

40 ml

Dilute 1+20 with 800 ml redistilled water to make Wash Buffer

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### Substrate TMB

*Note! Light sensitive!*

1 vial

22 ml

Ready for use

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### Stop Solution

0.5 M H<sub>2</sub>SO<sub>4</sub>

1 vial

7 ml

Ready for use

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## Preparation of Enzyme Conjugate

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

Number of strips	Enzyme Conjugate	Enzyme Conjugate buffer
4 strips	200 $\mu$ l	2 ml
6 strips	300 $\mu$ l	3 ml
12 strips (1 plate)	600 $\mu$ l	6 ml

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

## REAGENTS FOR 10 X 96 KIT

Each Mercodia Mouse Insulin ELISA kit (10-1149-10) 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.

<b>Coated Plate</b> (mouse monoclonal anti-insulin)	10 plates	à 96 wells	Ready for use 8-wells strips
For unused micro plate wells, completely reseal the bag using adhesive tape and use within two months.			
<b>Calibrators</b> Concentration indicated on vial label.	5 vials	1000 µl	Lyophilized Add 1.0 ml redistilled water per vial.
Use within 1 week. For storage longer than 1 week, store at –20°C.			
<b>Calibrator 0</b> Colour coded yellow	1 vial	5 ml	Ready for use
<b>Enzyme Conjugate 11X</b> (Peroxidase conjugated mouse monoclonal anti-insulin, 4.4 µg/l)	1 vial	6 ml	Preparation, see below
<b>Enzyme Conjugate Buffer</b> Colour coded blue	1 bottle	60 ml	Ready for use
<b>Wash Buffer 21X</b>	1 bottle	400 ml	Dilute 1+20 with 8000 ml redistilled water to make Wash Buffer
<b>Substrate TMB</b> <i>Note! Light sensitive!</i>	1 bottle	220 ml	Ready for use
<b>Stop Solution</b> 0.5 M H <sub>2</sub> SO <sub>4</sub>	1 bottle	70 ml	Ready for use

## Preparation of Enzyme Conjugate

Prepare the needed volume of Enzyme Conjugate 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
4 strips	200 $\mu$ l	2 ml
6 strips	300 $\mu$ l	3 ml
1 plate	600 $\mu$ l	6 ml
2 plates	1200 $\mu$ l	12 ml
3 plates	1800 $\mu$ l	18 ml
5 plates	3000 $\mu$ l	30 ml
10 plates	6000 $\mu$ l	60 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
4 strips	12 ml	240 ml
6 strips	20 ml	400 ml
1 plate	35 ml	700 ml
2 plates	70 ml	1400 ml
3 plates	110 ml	2200 ml
5 plates	180 ml	3600 ml
10 plates	350 ml	7000 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 $\mu$ l	–
2 Unknowns	–	25 $\mu$ l
3 Enzyme Conjugate	50 $\mu$ l	50 $\mu$ 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 $\mu$ 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 $\mu$ l	200 $\mu$ l
7 Incubate for 15 minutes		
8 Stop Solution	50 $\mu$ l	50 $\mu$ 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.		

## 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

### Data reduction

Use cubic spline regression for calculating the calibration curve. Calibrator 0 should not be included in the calculation.

### Manual Calculation

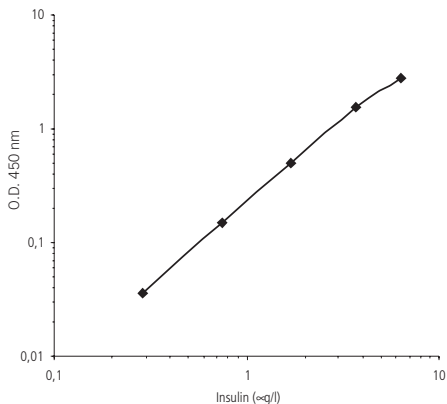
1. Plot the absorbance values obtained for the Calibrators, except Calibrator 0, against the insulin concentration on a log-log or lin-log paper and construct a Calibrator curve.
2. Read the concentration of the unknown samples from the Calibrator curve.

### Example of results

Wells	Identity	A <sub>450</sub>	Mean conc. µg/l
1A–B	0 Calibrator	0.073/0.074	
1C–D	0.188 µg/l Calibrator	0.091/0.093	
1E–F	0.5 µg/l Calibrator	0.148/0.152	
1G–H	1.25 µg/l Calibrator	0.382/0.384	
2A–B	3.75 µg/l Calibrator	1.434/1.466	
2C–D	6.9 µg/l Calibrator	2.714/2.646	
2E–F	Unknown 1	0.269/0.273	0.92
2G–H	Unknown 2	0.564/0.564	1.72
3A–B	Unknown 3	1.553/1.578	4.02

## Calibration curve

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



## EXPECTED VALUES

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

## Conversion factor

1 µg corresponds to 174 pmol:

Example:

µg/l	0.188	0.5	1.25	3.75	6.9
pmol/l	33	87	218	652	1200

## LIMITATIONS OF THE PROCEDURE

### Performance limitations

Grossly lipemic, icteric or hemolysed samples do not interfere in the assay.

## PERFORMANCE CHARACTERISTICS

### Detection limit

The detection limit is  $\leq 0.10 \mu\text{g/l}$  calculated as two standard deviations above the zero Calibrator.

### Hook effect

Samples with a concentration of up to at least  $345 \mu\text{g/l}$  can be measured without giving falsely low results.

### Recovery

Recovery upon addition is 108%.

### Precision

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

Sample	Mean value $\mu\text{g/l}$	Coefficient of variation		
		within assay %	between assay %	total assay %
1	0.955	2.9	6.3	7.0
2	1.74	2.2	4.9	5.4
3	4.16	1.8	4.0	4.4

## 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 authorised distributors, in such event, shall not be liable for damages indirect or consequential.

**SUMMARY PROTOCOL SHEET**  
**Mercodia Mouse Insulin ELISA**

Add Calibrators and samples	25 $\mu$ l
Add Conjugate solution	50 $\mu$ l
Incubate	2 hours at 18–25°C on a shaker
Wash	6 times
Add Substrate TMB	200 $\mu$ l
Incubate	15 minutes
Add Stop Solution	50 $\mu$ l <i>Shake for 5 seconds to ensure mixing</i>
Measure $A_{450}$	