Mercodia On-demand IAPP ELISA

Directions for Use

REF 10-1352-01

Reagents for 96 determinations

For Research Use Only
Not for Use in Diagnostic Procedures

Mercodia AB
Sylveniusgatan 8A
SE-754 50 Uppsala
Sweden

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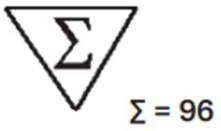
Explanation of symbols used on labels

	Reagents for 96 determinations
	Use-by date
	Temperature limit
	Batch code
	Catalogue number
	Consult electronic Instructions for Use
	Manufacturer

Intended use

The Mercodia On-demand IAPP ELISA provides a method for the quantitative determination of IAPP (islet amyloid polypeptide) in human plasma samples.

Principle of the procedure

Mercodia On-demand IAPP ELISA is a solid phase two-site enzyme immunoassay based on the sandwich technique, in which two monoclonal antibodies are directed against separate antigenic determinants on the IAPP molecule. During the first incubation IAPP in the sample reacts with anti-IAPP antibodies bound to microplate wells. After washing, peroxidase conjugated anti-IAPP antibodies are added. After a second incubation and a simple washing step, the bound conjugate is detected by reaction with the chemiluminescent substrate. A luminescence plate reader is used to read the intensity of light generated.

Warnings and precautions

- All samples should be handled as of capable of transmitting infections.
- Each well can only be used once.
- Do not mix components from different lots.
- Dispose of used components according to local regulations for biohazardous waste.

For safety information, please consult the safety data sheets (SDS) available at our website https://www.mercodia.com/technical-library/.

Material required but not provided

- Pipettes with appropriate volumes (repeating pipettes preferred for addition of enzyme conjugate 1X solution and substrate working solution)
- Tubes, beakers, and cylinders for reagent preparation
- Redistilled water
- Magnetic stirrer
- Vortex mixer
- Microplate reader for chemiluminescence
- Microplate orbital shaker (700-900 revolutions per minute)
- Microplate washing device with overflow function (recommended but not required)

Reagents

Mercodia On-demand IAPP ELISA contains reagents for 96 wells, sufficient for 39 samples, one negative control, anchor points and one calibrator curve in duplicates. 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 Rabbit monoclonal anti-IAPP	1 plate	96 wells 8-well strips	Ready for use
Calibrator Stock Solution	1 vial	1000 µL	Lyophilized. Reconstitute in 1000 µL redistilled water before use
Calibrator Dilution Buffer Color coded yellow Negative control	1 vial	10 mL	Ready for use
Assay Buffer Color coded red	1 vial	15 mL	Ready for use
Enzyme Conjugate 11X Peroxidase conjugated rabbit monoclonal anti-IAPP	1 vial	1.1 mL	Prepartation, see below
Enzyme Conjugate Buffer Color coded blue	1 vial	11 mL	Ready for use
Wash buffer 21X Storage after dilution, 2-8°C for 2 months	1 bottle	50 mL	Dilute with 1000 mL redistilled water to make wash buffer 1X solution
Substrate NL Ultra A Colorless solution	1 vial	2 mL	Preparation, see below
Substrate NL Ultra B Colorless solution	1 vial	4 mL	Preparation, see below

Preparation of calibrators and anchor points

The lyophilized Calibrator Stock Solution is reconstituted with 1000 µL redistilled water. Mix thoroughly. The reconstituted Calibrator Stock Solution has a concentration of 300 pM and may be used as the optional Anchor Point High (APH).

The Calibrator Stock Solution is diluted to prepare the calibrators and the optional Anchor Point Low (APL), according to the serial dilution procedure described in Figure 1. Mix each dilution thoroughly before proceeding to the next dilution step. The optional anchor points are used only to support curve fitting and are not part of the measuring range, which is defined by Calibrator 1–6.

Keep the Calibrator Stock Solution, prepared calibrators, and optional anchor points on ice during preparation.

The Calibrator Stock Solution, prepared calibrators, and optional anchor points are intended for single use only. Stability after reconstitution or preparation has not been established. Storage of these components after use is therefore not recommended. Discard any remaining material after the assay run.

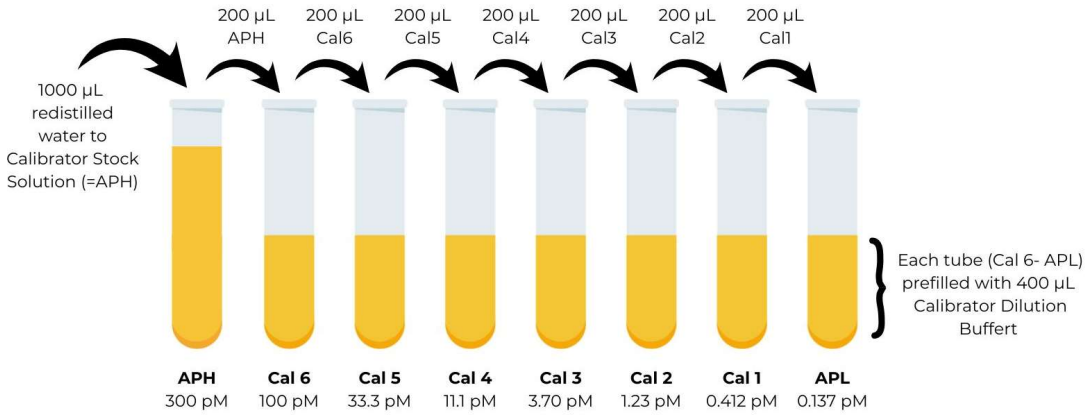


Figure 1. Serial dilution procedure for preparation of calibrators and optional anchor points from the reconstituted Calibrator Stock Solution. APH = Anchor Point High, APL = Anchor Point Low, Cal = Calibrator.

Specimen handling

Store plasma samples at -80 °C and avoid freeze-thaw cycles. Avoid storing samples at room temperature or 2-8 °C. It is recommended to keep samples on ice when thawing them and while preparing the assay.

Preparation of samples

No dilution is normally required, however, samples above the obtained value of Calibrator 6 should be diluted with Assay buffer. *Note!* 9.6 mL Assay Buffer is needed to run a full plate of calibrators and samples, and therefore it is important to not use more than 5 mL Assay Buffer for dilution of samples.

Test procedure

All reagents must be brought to room temperature before use. Prepare a calibrator curve for each assay run.

1. Prepare Wash Buffer 1X by diluting Wash Buffer 21X with 1000 mL redistilled water.
2. Prepare the Enzyme Conjugate 1X solution by pouring all the blue Enzyme Conjugate Buffer into the Enzyme Conjugate 11X vial. Mix gently.
3. Prepare the calibration curve by reconstituting Calibrator Stock Solution with 1000 µL redistilled water, and further serial dilution in Calibrator Dilution Buffer (see section Preparation of calibrators and anchor points on page 5). Keep all calibrator curve components on ice during preparation.
4. Pipette 50 µL each of Calibrators, anchor points (optional), negative control (Calibrator Dilution Buffer) and samples into appropriate wells.
5. Pipette 100 µL of Assay Buffer into each well.
6. Incubate on plate shaker (700-900 rpm) 1h at room temperature (18-25ºC).
7. 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 buffer 1X solution, tap firmly several times against absorbent paper to remove excess liquid. Repeat 5 times for a total of 6 cycles.
8. Pipette 100 µL of Enzyme Conjugate 1X solution into each well.
9. Incubate on plate shaker (700-900 rpm) 1h at room temperature (18-25ºC).
10. Prepare the needed volume of substrate working solution by pouring Substrate NL Ultra B into the vial with Substrate NL Ultra A. Mix gently. *Note!* Make sure to protect the solution from light and be careful not to contaminate the substrate working solution with enzyme conjugate solution.
11. Wash as described in step 7.
12. Add 50 µL Substrate working solution into each well.
13. Incubate in the dark for 15 minutes at room temperature (18-25 °C), without shaking.
14. Use a microplate reader for chemiluminescence. Measure all visible light (glow) with an integration time of 1.0 second. No filter is needed. Use settings for a 96 well plate with flat bottom. Instrument settings should be used according to the manufacturer's instructions.
15. Apply curve fitting directly on raw data (RLU). Preferably use 5-parametric logistic regression with weighing using $1/Y^2$.

Internal quality controls

Internal plasma pools with low, intermediate and high IAPP 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 and/or reconstitution dates of kit components, relative light units (RLU) values for the blank and Calibrators and concentration values for the controls.

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

Calculation of results

The concentration of IAPP is obtained by plotting the relative light units (RLU) of the Calibrators and optional Anchor Points versus their respective concentration. It is important to use an appropriate curve fitting model that represents the true dose-response relationship to get accurate results. It is every laboratory's responsibility to validate the functionality of the chosen curve fitting model and software used.

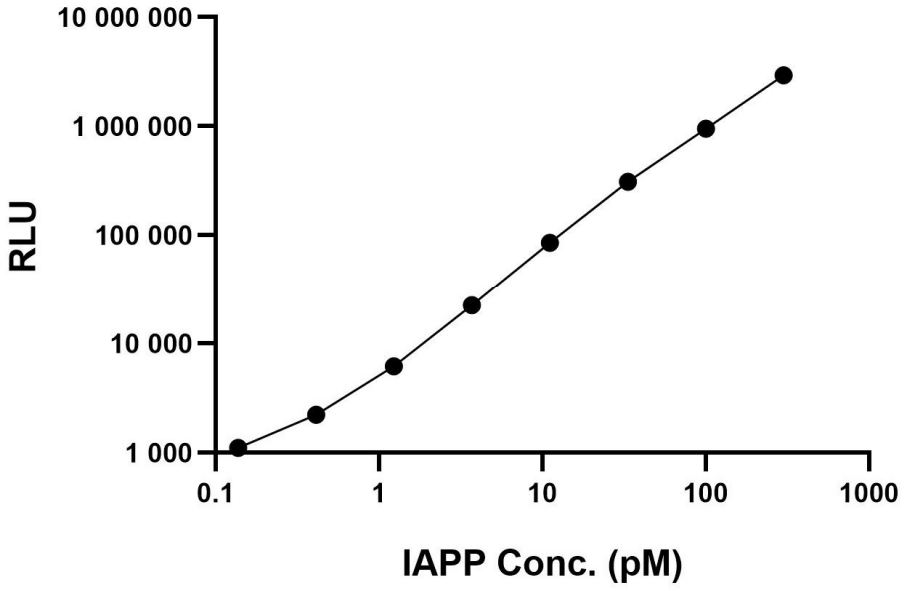
The Mercodia On-demand IAPP ELISA is developed using Infinite M PLEX (Tecan) with the software Magellan Pro Tracker v 7.5 (Tecan) with Five Parameter Logistic (5PL) and automatic weighting using $1/Y^2$.

Example of results

Wells	Identity	RLU	Mean conc. pM
1A-B	Negative control	856/876	-
1C-D	APL (0.137 pM)	1196/1260	-
1E-F	Cal1 (0.412 pM)	2352/2472	-
1G-H	Cal2 (1.23 pM)	6971/6534	-
2A-B	Cal3 (3.70 pM)	24711/23018	-
2C-D	Cal4 (11.1 pM)	87547/83405	-
2E-F	Cal5 (33.3 pM)	294482/291733	-
2G-H	Cal6 (100 pM)	945039/939408	-
3A-B	APH (300 pM)	2784022/2709308	-
3C-D	Sample 1	54709/54061	8.00
3E-F	Sample 2	79678/77241	10.9
3G-H	Sample 3	139192/139322	17.8

Example of calibrator curve

A typical calibrator curve is shown here, including APL, Calibrators 1–6, and APH. The anchor points, APL and APH, are optional and are used only to support curve fitting. They are not part of the measuring range. Do not use this curve to determine actual assay results.



Calibration

The concentration of the Calibrator Stock Solution was assigned based on a commercially sourced preparation of synthetic human IAPP (1-37).

Performance characteristics

Sensitivity

The limit of detection (LOD) is a calculated concentration corresponding to the average signal 3 standard deviations above the background (Calibrator buffer = Blank). The LOD was estimated to 0.197 pM.

Each laboratory should verify that the assay performance, including LOD and measuring range, is suitable under its own laboratory conditions.

Precision

P800 plasma samples were analyzed in duplicates over 4 different days, on one kit lot, one instrument system and by one laboratory technician.

		Coefficient of variation	
Sample	Mean value (pM)	Within-run (%)	Between-run (%)
Sample 1	7.7	0.2 - 1.4	4.9
Sample 2	10.6	0.1 - 3.8	2.9
Sample 3	19.4	0.1 - 1.6	5.5

Parallelism

Six human P800 plasma samples with different concentrations were diluted 1/2, 1/4, and 1/8. Mean recovery for parallelism is 106% (86-118%) with precision between samples within dilution series ranging from 5 to 11%.

Specificity

The following cross-reactions have been tested:

Substance	Cross-reaction (%)	Concentrations tested
Mouse IAPP	<0.01	100 pM and 100 nM
CGRP-1	<0.01	100 pM and 100 nM
CGRP-2	0.01	100 pM and 100 nM
GLP-1	0.01	100 pM and 100 nM
Glucagon	0.02	100 pM and 100 nM
Insulin	0.01	100 pM and 100 nM

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.

Summary of protocol sheet

Mercodia On-demand IAPP ELISA

Add Calibrators and samples	50 µL
Add Assay Buffer	100 µL
Incubate	1h at room temperature (18-25°C) on a plate shaker, 700-900 rpm
Wash plate with wash buffer 1X solution	700 µL, 6 times
Add Enzyme Conjugate 1X solution	100 µL
Incubate	1h at room temperature (18-25°C) on a plate shaker, 700-900 rpm
Wash plate with wash buffer 1X solution	700 µL, 6 times
Add substrate working solution	50 µL
Incubate	15 minutes in the dark at 18-25 °C (without shaking)
Measure chemiluminescence	1.0 s integration time (glow),

For full details see page 6.

For technical support please contact: support@mercodia.com