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User's Manual

Major Proglucagon Fragment (MPGF) ELISA Kit

REF **DEIAPL1211**

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

The Major Proglucagon Fragment (MPGF) enzyme linked immunosorbent assay (ELISA) kit provides materials for the quantitative measurement of MPGF in EDTA plasma and other biological fluids. This kit is intended for Research Use Only and is not for use in diagnostic or therapeutic procedures.

General Description

Major Proglucagon Fragment (MPGF) is an 86-amino acid hormone secreted from the pancreas[1-3]. MPGF is identified as the Carboxy terminal portion of proglucagon that contains two glucagon-related sequences. The MPGF sequence is highly conserved among mammals. Tissue specific processing of proglucagon in the pancreas releases MPGF. Intestinal processing of MPGF releases the Glucagon-Like Peptides 1 and 2 (GLP-1 and GLP-2)[1-5]. Measuring the circulating levels of MPGF will help in understanding the defective or abnormal metabolic pathways leading to diabetes and obesity.

Principles of Testing

The MPGF is a quantitative three-step sandwich type immunoassay. In the first step Calibrators, Controls and Unknown Samples are added to MPGF antibody coated microtiter wells and incubated. After the first incubation and washing, the wells are incubated with biotinylated MPGF antibody. After the second incubation and washing, the wells are incubated with streptavidin horseradish peroxidase conjugate (SHRP). After the third incubation and washing step, the wells are incubated with substrate solution (TMB). After TMB incubation, an acidic stopping solution is added. In principle, the antibody-biotin conjugate binds to the solid phase antibody-antigen complex which in turn binds to the streptavidin-enzyme conjugate. The antibody-antigen-biotin conjugate-SHRP complex bound to the well is detected by enzyme-substrate reaction. The degree of enzymatic turnover of the substrate is determined by dual wavelength absorbance measurement at 450 nm as primary test filter and 630 nm as reference filter. The absorbance measured is directly proportional to the concentration of MPGF in the samples and calibrators.

Reagents And Materials Provided

MPGF Calibrator A/Sample Diluent

One bottle, 3 mL, labeled MPGF Cal. A/ Sample Diluent, containing 0 ng/mL MPGF in protein based buffer and Pro-Clean 400. Store unopened at 2-8°C until the expiration date.

MPGF Calibrators B-F (Lyophilized)

Five vials, labeled B-F, containing concentrations of approximately 0.05 - 3 ng/mL MPGF in protein based buffer and Pro-Clean 400. Refer to calibration card for exact concentrations. Store unopened at 2 to 8°C until the expiration date. Reconstitute calibrators B-F with 1 mL deionized water. Solubilize, mix well and use after reconstitution. Aliquot and freeze in plastic vials immediately for multiple use and discard after the run. Avoid repeated freeze thaws. The MPGF concentration in the MPGF calibrators is traceable to the manufacturer's working calibrators. Values assigned by other methodologies may be different. Such differences, if present, may be caused by inter-method bias.

MPGF Controls I & II

Two vials, labeled Levels I and II containing low and high MPGF concentrations in protein based buffer and Pro-Clean 400. Refer to calibration card for exact concentrations. Store unopened at 2 to 8°C until the expiration date. Reconstitute control Levels I and II with 1 mL deionized water. Solubilize, mix well and use after reconstitution. Aliquot and Freeze immediately in plastic vials for multiple use and discard after running. Avoid repeated freeze thaws.

Anti-MPGF Coated Microtitration strips

One strip holder, containing 12 strips and 96 microtitration wells with MPGF antibody immobilized to the inside wall of each well. Store at 2-8°C until expiration date in the resealable pouch with a desiccant to protect from moisture.

Proglucagon Assay Buffer

One bottle, 8 mL, containing a buffer solution with a non-mercury preservative. Store at 2-8°C until expiration date.

MPGF Biotin Conjugate Concentrate (50X)

One vial, 0.4 mL, containing detection antibody biotin in a protein-based buffer with a non-mercury preservative. Dilute prior to use in GLP Conjugate Diluent. Store at 2-8°C until expiration date.

GLP Conjugate Diluent

One bottle, 12 mL, containing a protein-based buffer with a non-mercury preservative. Store at 2-8°C until expiration date.

MPGF Streptavidin-Enzyme Conjugate-Ready-to-Use(RTU)

One bottle, 12 mL, containing streptavidin-HRP (horseradish peroxidase) in a protein-based buffer and a non-mercury preservative. Store undiluted at 28°C until expiration date.

TMB Chromogen Solution

One bottle, 12 mL, containing a solution of tetramethylbenzidine (TMB) in buffer with hydrogen peroxide. Store at 2-8°C until expiration date.

Stopping Solution

One bottle, 12 mL, containing 0.2 M sulfuric acid. Store at 2 to 30°C until expiration date.

Wash Concentrate A

One bottle, 60 mL, containing buffered saline with a nonionic detergent. Store at 2 to 30°C until expiration date. Dilute 25-fold with deionized water prior to use.

Materials Required But Not Supplied

1. Microtitration plate reader capable of absorbance measurement at 450 nm, 405 nm and 630 nm.
2. Microplate shaker.
3. Microplate washer.
4. Semi-automated/manual precision pipette to deliver 10-250 µL.
5. Vortex mixer.
6. Deionized water.

7. Disposable 12 x 75 mm culture tubes.

Storage

Store at 2-8°C.

Specimen Collection And Preparation

a) K_2EDTA Plasma is the recommended sample type.

b) Sample handling, processing, and storage requirements depend on the brand of blood collection tube that you use. Please reference the manufacturer's instructions for guidance. Each laboratory should determine the acceptability of its own blood collection tubes and serum separation products.

c) Samples may be stored at 4°C if assayed within 24 hours; otherwise samples must be stored at -20°C or -80°C to avoid loss of bioactivity and contamination.

d) Avoid assaying lipemic, hemolyzed or icteric samples.

e) Avoid repeated freezing and thawing of samples. Thaw samples no more than 3 times. For shipping, place specimens in leak proof containers in biohazard specimen bags with appropriate specimen identification and test requisition information in the outside pocket of the biohazard specimen bag. Follow DOT and IATA requirements when shipping specimens

Reagent Preparation

1. MPGF Calibrators B-F and MPGF Controls I & II: Tap and reconstitute MPGF Calibrator B-F and MPGF Controls I & II each with 1 mL deionized water. Solubilize, mix well and use after reconstitution. To homogenize, leave for 15 min before use.
2. Wash Solution: Dilute wash concentrate 25-fold with deionized water. The wash solution is stable for one month at room temperature when stored in a tightly sealed bottle.
3. Microtitration Wells: Select the number of coated wells required for the assay. The remaining unused wells should be placed in the resealable pouch with a desiccant. The pouch must be resealed to protect from moisture.
4. MPGF Antibody-Biotin Conjugate Solution: The MPGF Antibody-Biotin Conjugate Concentrate should be diluted at a ratio of 1-part conjugate to 50 parts of GLP Conjugate Diluent, according to the number of wells used. If an entire plate is to be used pipet exactly 220 µL of the Concentrate in to 11 mL of the diluent.

Assay Procedure

Procedure Notes

1. A thorough understanding of this package insert is necessary for successful use of the MPGF assay. It is the customer's responsibility to validate the assay for their use. Accurate results will only be obtained by using precise laboratory techniques and following the package insert.
2. A calibration curve must be included with each assay.
3. Bring all kit reagents to room temperature before use. Thoroughly mix the reagents before use by gentle inversion. Do not mix various lots of any kit component and do not use any component beyond the

expiration date.

4. Use a clean disposable pipette tip for each reagent, calibrator, control or sample. Avoid microbial contamination of reagents, contamination of the substrate solutions with the HRP conjugates. The enzyme used as the label is inactivated by oxygen, and is highly sensitive to microbial contamination, sodium azide, hypochlorous acid and aromatic chlorohydrocarbons often found in laboratory water supplies. Use deionized water.
5. Incomplete washing will adversely affect the outcome and assay precision. Care should be taken to add TMB into the wells to minimize potential assay drift due to variation in the TMB incubation time. Avoid exposure of the reagents to excessive heat or direct sunlight.

Assay Procedure

Allow all specimens and reagents to reach room temperature and mix thoroughly by gentle inversion before use. Calibrators, Controls, and Unknowns should be assayed in duplicate.

1. Label the microtitration strips to be used.
2. Pipette 25 µL of the Calibrators (Cal A-F), Control and Unknowns to the appropriate wells.
3. Add 50 µL of the Proglucagon Assay Buffer to each well using a repeater pipette.
4. Incubate the plate, shaking at a fast speed (600-800 rpm) on an orbital microplate shaker, for 60 minutes at room temperature (23±2°C).
5. During the last 20-30 minutes of incubation, prepare the MPGF Antibody-Biotin Conjugate Solution by diluting the MPGF Biotin Conjugate Concentrate in GLP Conjugate Diluent as described under the Preparation of the Reagents section of this package insert.
6. Aspirate and wash each strip 5 times with Wash Solution using an automatic microplate washer.
7. Add 100 µL of the Antibody-Biotin Conjugate Solution to each well using a repeater pipette.
8. Incubate the plate, shaking at a fast speed (600-800 rpm) on an orbital microplate shaker, for 60 minutes at room temperature (23±2°C).
9. Aspirate and wash each strip 5 times with the Wash Solution using an automatic microplate washer.
10. Add 100 µL of the Streptavidin-Enzyme Conjugate-RTU to each well using a repeater pipette.
11. Incubate the plate, shaking at a fast speed (600-800 rpm) on an orbital microplate shaker, for 30 minutes at room temperature (23±2°C).
12. Aspirate and wash each strip 5 times with the Wash Solution using an automatic microplate washer.
13. Add 100 µL of the TMB chromogen solution to each well using a precision pipette. Avoid exposure to direct sunlight.
14. Incubate the wells, shaking at 600-800 rpm on an orbital microplate shaker, for 8-10 min at room temperature (23±2°C). **NOTE:** Visually monitor the color development to optimize the incubation time.
15. Add 100 µL of the stopping solution to each well using a precision pipette. Read the absorbance of the solution in the wells within 20 minutes, using a microplate reader set to 450 nm.

NOTE: Zero calibrator should be programmed as "Blank" while reading the optical density. If instrument has a wavelength correction, set the instrument to dual wavelength measurement at 450 nm with background wavelength correction at 630 nm.

Quality Control

1. Each laboratory should establish mean values and acceptable ranges to assure proper performance.
2. MPGF ELISA controls or other commercial controls should fall within established confidence limits.
3. The confidence limits for MPGF controls are printed on the Calibration Card.
4. A full calibration curve, low and high-level controls, should be included in each assay.
5. TMB should be colorless. Development of any color may indicate reagent contamination or instability.

Calculation

NOTE: The results in this package insert were calculated by plotting the log optical density (OD) data on the y-axis and log MPGF concentration on x-axis using a cubic regression curve-fit. Alternatively, linear regression curvefit can be used. Other data reduction methods may give slightly different results.

1. Calculate the mean optical density (OD) for each calibrator, Control, or Unknown.
2. Optimum results can be obtained at incubation temperature of (23 ± 2°C).
3. Plot the log of the mean OD readings for each of the Calibrators along the y-axis versus log of the MPGF concentrations in ng/mL along the x-axis, using a cubic regression curve-fit.
4. Determine the MPGF concentrations of the Controls and unknowns from the calibration curve by matching their mean OD readings with the corresponding MPGF concentrations.
5. Any sample reading higher than the highest Calibrator should be appropriately diluted with the 0 ng/mL (Cal. A / Sample Diluent) and re-assayed.
6. Any sample reading lower than the analytical sensitivity should be reported as such.
7. Multiply the value by a dilution factor, if applicable.

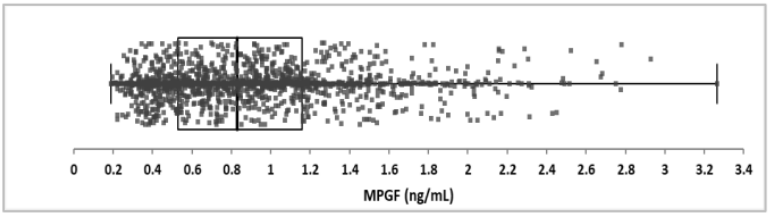
Typical Standard Curve

Well Number	Well Contents	Mean Absorbance	Conc. (ng/mL)
	Calibrators	0.038 (Blank)	0
A1, A2	A		
B1, B2	B	0.096	0.05
C1, C2	C	0.25	0.157
D1, D2	D	0.69	0.45
E1, E2	E	1.89	1.4
F1, F2	F	3.28	3

CAUTION: The above data must not be employed in lieu of data obtained by the user in the laboratory.

Reference Values

Expected MPGF concentration in undifferentiated population (diabetic and non-diabetic) was calculated by evaluating 1534 samples in Ansh Labs MPGF ELISA. The frequency distribution was calculated using Analyse-It® for Microsoft Excel and is shown below.



n	MPGF (ng/mL)		
	Mean	Median	Range
1534	0.9	0.8	0.2 - 3.3

Quantile	MPGF (ng/mL)
0.100	0.4
0.200	0.5
0.300	0.6
0.400	0.7
0.500	0.8
0.600	1.0
0.700	1.1
0.800	1.3
0.900	1.5

NOTE: It is recommended that each laboratory should determine the reference range(s) for its own patient population. The results of this assay should be used in conjunction with other relevant and applicable clinical information.

Sensitivity

The analytical sensitivity in the MPGF ELISA assay, as calculated by the interpolation of mean plus two standard deviations of 16 replicates of calibrator A (0 ng/mL) and calibrator B (0.05 ng/mL), is 0.003 ng/mL.

Specificity

The MPGF sequence is highly conserved among mammals. Antibody pair used in the assay detects human MPGF and cross-reactivity to other closely related analytes is listed below.

Cross-Reactant	Concentration	% Cross-Reactivity
Oxyntomodulin	100 ng/mL	ND
Glucagon	100 ng/mL	ND
GLP-1(1-36)	100 ng/mL	ND
GLP-1 (7-36)	100 ng/mL	ND
GLP-1 (9-36)	100 ng/mL	ND
GLP-2 (1-34)	100 ng/mL	ND
GRPP	100 ng/mL	ND
MPGF-1	10 ng/mL	100%
MPGF-2	10 ng/mL	1.7%
Insulin	10 ng/mL	ND
C-Peptide	10 ng/mL	ND
Thyroglobulin	10 ng/mL	ND
Glicentin	100 ng/mL	ND

Species Immunoreactivity

Antibody pair used in MPGF detects Goat, Ovine, Canine, Bovine, Porcine, Squirrel Monkey and Vervet Monkey.

Linearity

Human EDTA plasma samples and calibrator F containing various MPGF levels were diluted with Calibrator A/sample diluent. The % recovery on individual samples is represented in the following table.

Sample ID	Dilution Factor	Expected Conc. (ng/mL)	Observed Conc. (ng/mL)	% Recovery
Calibrator F	Neat	3.000	NA	NA
	1:2	1.500	1.507	100%
	1:4	0.750	0.745	99%
	1:8	0.375	0.375	100%
	1:16	0.188	0.187	100%
	1:32	0.094	0.93	99%
Sample-1	Neat	0.180	NA	NA
	1:2	0.090	0.107	119%
	1:4	0.045	0.049	109%
Sample 2	Neat	0.722	NA	NA
	1:2	0.361	0.361	100%
	1:4	0.181	0.192	106%
	1:8	0.090	0.097	107%
	1:16	0.045	0.044	98%

Recovery

Known amounts of MPGF antigen were added to three serum samples containing different levels of endogenous MPGF. The concentration of MPGF was determined before and after the addition of exogenous MPGF and the percent recovery was calculated.

Sample ID	Endogenous Value in ng/mL	Expected in ng/mL	Observed in ng/mL	%Recovery	Average %Recovery
1	0.744	0.769	0.766	100%	96%
		0.794	0.764	96%	
		0.819	0.751	92%	
2	0.374	0.417	0.408	98%	100%
		0.461	0.463	100%	
		0.504	0.516	102%	
3	0.578	0.611	0.595	97%	95%
		0.644	0.613	95%	
		0.678	0.623	92%	

Reproducibility

Reproducibility of the MPGF assay was determined in a study using two kit controls. The study included a total of twenty assays, replicates of six per assay (n=120). Representative data were calculated and are presented in the following table.

Sample	Mean Conc.	Within Run		Between Run		Total	
	(ng/mL)	SD	%CV	SD	%CV	SD	%CV
Control I	0.46	0.03	5.47%	0.03	5.54%	0.04	7.79%
Control II	1.37	0.09	6.40%	0.06	4.54%	0.11	7.84%

Interferences

When potential interferents (Hemoglobin, biotin, bilirubin, and intralipids) were added at least two times their physiological concentration to control sample, MPGF concentration were within ± 10% of the control as represented in the following table. This study was based on NCCLS EP-7.

Interferent	Interferent Dose	Sample MPGF (ng/mL)	Dosed Sample MPGF (ng/mL)	% Difference to Reference
Hemoglobin	1 mg/mL	0.85	0.83	-3.2
	0.5 mg/mL	0.88	0.89	1.7
	0.1 mg/mL	0.89	0.92	3.2
Hemoglobin	1 mg/mL	0.52	0.54	4.0
	0.5 mg/mL	0.57	0.56	-0.6
	0.1 mg/mL	0.57	0.57	0.1
Biotin	1200 ng/mL	0.80	0.80	-1.1
	600 ng/mL	0.88	0.85	-3.5
	200 ng/mL	0.92	0.91	-1.6
Biotin	1200 ng/mL	0.51	0.54	5.0
	600 ng/mL	0.56	0.56	-0.4
	200 ng/mL	0.59	0.61	3.8
Intralipids	20 mg/mL	0.80	0.81	2.1
	10 mg/mL	0.81	0.83	2.0
	5 mg/mL	0.83	0.86	4.2
Intralipids	20 mg/mL	0.52	0.57	8.0
	10 mg/mL	0.56	0.53	-4.6
	5 mg/mL	0.58	0.61	4.9
Bilirubin	0.66 mg/mL	0.66	0.64	-3.1
	0.66 mg/mL	0.42	0.40	-2.8

Hook Effect

There is no high-dose hook effect at MPGF concentrations up to 18.4 ng/mL.

Precautions

For Research Use Only. Not for use in diagnostic procedures. The following precautions should be observed:

a) Follow good laboratory practice.

b) Use personal protective equipment. Wear lab coats and disposable gloves when handling immunoassay materials.

c) Handle and dispose of all reagents and material in compliance with applicable regulations.

WARNING:Potential Biohazardous Material

This reagent may contain some animal source material (e.g. BSA) or materials used in conjunction with animal source materials. Handle all reagents and patient samples at a Biosafety Level 2, as recommended for any potentially infectious human material in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories," 5th Edition, 2007.[6]

WARNING: Potential Chemical Hazard

Some reagents in this kit contain Pro-Clean 400 and Sodium azide[7] as a preservative. Pro-Clean 400 and Sodium azide in concentrated amounts are irritants to skin and mucous membranes.

Limitations

The reagents supplied in this kit are optimized to measure MPGF levels in human EDTA plasma. If there is

evidence of microbial contamination or excessive turbidity in a reagent, discard the vial. For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the samples.[8]

References

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