INSTRUCTION MANUAL

REF **3950**

May 15, 2017

Pancreatitis GP2

- 96 determinations -

CE

IVD *In vitro* diagnostic device

Enzyme immunoassay for the quantitative determination of glycoprotein 2 in human serum

REF	Catalogue number	**LOT**	Batch code
	Consult accompanying documents		Manufactured by
	Temperature limitation		Use by
	Consult operating instruction		Biological risk

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manufactured under the licence of patents including
US 8058019, EP 2126582, JP 2010519180, CA 2674021, AU 2008209176, ZL 2008800030495, 10-1283710

INTENDED USE

Pancreatitis GP2 is used for the quantitative determination of glycoprotein 2 isoform alpha (GP2a) in human serum for the diagnosis of acute pancreatitis.

The serological diagnosis of acute pancreatitis, a major cause for hospitalization in case of acute abdominal pain, is still a laboratory challenge. The incidence of acute pancreatitis ranges from 17.5 to 73.4 cases per 100,000 individuals globally. Although the pathophysiology of acute pancreatitis is not understood entirely yet, it is now widely acknowledged that premature intra-pancreatic activation of proenzymes in particular trypsinogen stored in zymogen granules (ZG) plays an important role. Thus, acute pancreatitis onset is characterized by acinar cell injury resulting in an impaired polarity of proenzyme secretion and basolateral release of ZG contents.

A well-characterized animal model of acute pancreatitis revealed elevated major zymogen granule membrane glycoprotein 2 (GP2) levels as a potential serum marker. Based on this model, an ELISA for the detection of acute pancreatitis-specific GP2 has been developed and the diagnostic and prognostic value of serum GP2 levels in a large cohort of patients with acute pancreatitis and extensive disease-control groups have been investigated. The alpha isoform of GP2 is a specific marker for acute pancreatitis and enables the differentiation to chronic pancreatitis and pancreatic neoplasms. Furthermore, GP2a can be used to predict the mortality of the disease which renders GP2a a promising diagnostic and prognostic acute pancreatitis marker.

Roggenbuck D, Goihl A, Hanack K, Holzlöhner P, Hentschel C, Veiczi M, Schierack P, Reinhold D, Schulz HU: Serological diagnosis and prognosis of severe acute pancreatitis by analysis of serum glycoprotein 2. Clin Chem Lab Med. 2016 Nov 12. pii: /j/cclm.ahead-of-print/cclm-2016-0797/cclm-2016-0797.xml. doi

Lowe AW, Luthen RE, Wong SM, Grendell JH: The level of the zymogen granule protein GP2 is elevated in a rat model for acute pancreatitis. Gastroenterology. 1994 Dec;107(6):1819-27.

PRINCIPLE OF THE TEST

Pancreatitis GP2 is an enzyme immunoassay for the quantitative determination of glycoprotein 2 in human serum.

The GP2 in the calibrators, positive control, and patient samples react with the monoclonal antibody immobilized on the solid phase of microtiter plates. After an incubation period of 60 min at room temperature (18…25°C), unbound serum components are removed by a wash step.

The bound GP2 reacts specifically with polyclonal anti-GP2-antibodies conjugated to horseradish peroxidase (HRP) within the incubation period of 30 min at room temperature. Excessive conjugate is separated from the solid-phase immune complexes by the following wash step.

HRP converts the colorless substrate solution of 3,3',5,5'-tetramethyl-benzidine (TMB) added into a blue product. This enzyme reaction is stopped by dispensing an acidic solution (H_2SO_4) into the wells after 15 min at room temperature turning the solution from blue to yellow.

The optical density (OD) of the solution at 450 nm is directly proportional to the amount of GP2 bound. The standard curve is established by plotting the GP2 concentrations of the calibrators (x-axis) and their corresponding OD values (y-axis) measured. The concentration of GP2 of the specimen is directly read off the standard curve.

PATIENT SAMPLES

Specimen collection and storage

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Lipaemic, hemolytic and contaminated samples should not be used.

Samples are stable up to 3 days at 2-8°C, for extended storage freeze at -20 °C. Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at - 20 °C.

Preparation before use

Allow samples to reach room temperature prior to assay. Take care to agitate serum samples gently in order to ensure homogeneity.

Note: ***Patient samples have to be used undiluted.***

TEST COMPONENTS FOR 96 DETERMINATIONS

A Ag 96	**Microtiter plate**, 12 breakable strips per 8 wells; coated with monoclonal anti-glycoprotein 2 antibody	1 vacuum sealed with desiccant
B BUF WASH 10x	**Concentrated wash buffer** sufficient for 1000 ml solution	100 ml concentrate capped white
D CONJ	**Conjugate** containing anti-GP2 antibody (rabbit) coupled with HRP	15 ml ready for use capped red
E SOLN TMB	**Substrate** 3,3',5,5'-tetramethylbenzidine in citrate buffer containing hydrogen peroxide	15 ml ready for use capped blue
F H2SO4	**Stop solution** 0.25 M sulfuric acid 0.25M	15 ml ready for use capped yellow
0 - 4 CAL	**Calibrators** (diluted serum) conc.: 0.1, 0.4, 1, 3, 10 ng/ml GP2	1 ml each ready for use capped white
P CONTROL	**Control** (diluted serum) conc.: see leaflet enclosed +	1 ml ready for use capped red

Materials required in addition

- micropipette 100 - 1000 µl
- micropipette 10 - 100 µl
- multi-channel pipette 50 - 200 µl
- 8-channel wash comb with vacuum pump and waste bottle or microplate washer
- microplate reader with optical filters for 450 nm and 620 nm or 690 nm
- graduated cylinders
- tubes (2 ml) for sample preparation

Size and storage

Pancreatitis GP2 has been designed for 96 determinations.

The expiry date of each component is reported on its respective label that of the complete kit on the box labels.

Upon receipt, all components of the Pancreatitis GP2 have to be kept at 2 - 8 °C, preferably in the original kit box.

After opening all kit components are stable for at least 2 months, provided proper storage.

Preparation before use

Allow all components to reach room temperature prior to use in the assay.

The microtiter plate is vacuum-sealed in a foil with desiccant. The plate consists of a frame and strips with breakable wells. Allow the sealed microplate to reach room temperature before opening. Unused wells should be stored refrigerated and protected from moisture in the original cover carefully resealed.

Prepare a sufficient amount of wash solution by diluting the concentrated wash buffer 10 times (1 + 9) with de-ionized or distilled water.
For example, dilute 8 ml of the concentrate with 72 ml of distilled water. The wash solution prepared is stable up to 30 days at 2 - 8 °C.

Make sure the soak time of the wash buffer in the wells is at least 5 seconds per wash cycle.

Avoid exposure of the TMB substrate solution to light!

ASSAY PROCEDURE

- **Use undiluted sera.**
- **Avoid any time shift during pipetting of reagents and samples.**

1. Bring all reagents to room temperature (18…25°C) before use. Mix gently, avoid foam.
2. Dispense
   100 µl calibrators 0 - 4
   100 µl control P
   100 µl undiluted patient samples
   into the respective wells.
3. Seal plate, incubate **60 min** at room temperature.
4. Decant, then wash each well **three** times using **300 µl** wash solution (made of B).
5. Add **100 µl** of conjugate (D) solution to each well.
6. Seal plate, incubate **30 min** at room temperature.
7. Decant, then wash each well **three** times using **300 µl** wash solution (made of B).
8. Add **100 µl** of substrate (E) to each well.
9. Incubate **15 min** protected from light at room temperature.
10. Add **100 µl** of stop solution (F) to each well and mix gently.
11. Read the OD at **450 nm** versus 620 or 690 nm within **30 min** after adding the stop solution.

DATA PROCESSING

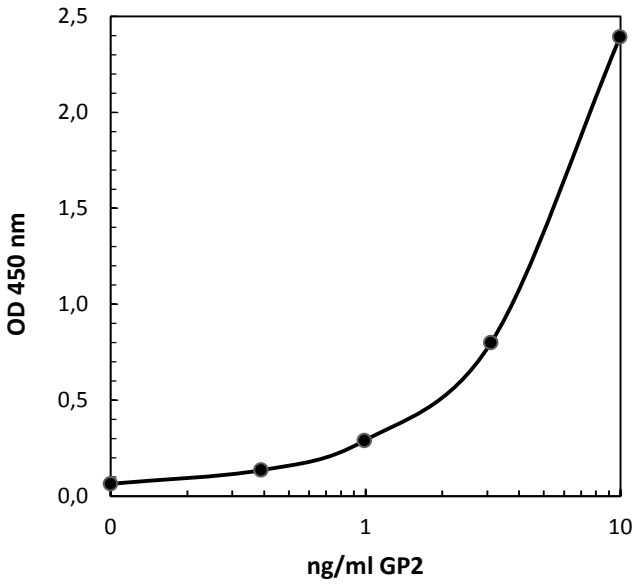
The standard curve is established by plotting the mean OD-values of the calibrators 0 - 4 on the ordinate, y-axis, (lin. scale) versus their respective GP2 concentrations on the abscissa, x-axis, (log. scale). GP2 concentrations of the unknown samples are directly read off in ng/ml against the respective OD values.

The evaluation of Pancreatitis GP2 may be achieved also with computer assisted analysis software integrated in the photometers.

Example of typical assay results

well	OD (a)	OD (b)	OD(mean)	ng/ml
Calibrator 0	0.065	0.065	0.065	**0.1**
Calibrator 1	0.136	0.134	0.135	**0.4**
Calibrator 2	0.294	0.286	0.290	**1**
Calibrator 3	0,812	0.787	0.800	**3**
Calibrator 4	2.391	2.393	2.392	**10**
Patient 1	0.429	0.422	0.426	**1.53**

TYPICAL STANDARD CURVE



Test validity

The test run is valid if:

- the mean OD of the calibrator 4 is ≥ 1.2
- Concentration of Control P see leaflet enclosed

If the above mentioned quality criteria are not met, repeat the test and make sure that the test procedure is followed correctly (incubation times and temperatures, sample and wash buffer dilution, wash steps etc.). In case of repeated failure of the quality criteria contact your supplier.

REFERENCE VALUES

Glycoprotein 2	ng/ml
Cut off	0.4

It is recommended that each laboratory establishes its own normal and pathological reference ranges for serum GP2 levels, as usually done for other diagnostic parameters, too. Therefore, the above mentioned reference values only provide a guide to values which might be expected.

Limitations of Method

Any clinical diagnosis should not be based on the results of in vitro diagnostic methods alone. Physicians are supposed to consider all clinical and laboratory findings possible to state a diagnosis.

CHARACTERISTIC ASSAY DATA

Limit of Detection

The limit of detection (LOD) of Pancreatitis GP2 was determined at 0.2 ng/ml.

Linearity

Linearity was evaluated by diluting a high dose sample with increasing amounts (from 0% to 100% in increments of 20%) of a sample that did not contain GP2a. R^2 value for linearity was determined to 0.99.

Recovery

Recovery was conducted by spiking human serum devoid of GP2a with recombinant GP2a. Recovery ranged from 92.3% - 113.6% for GP2a levels of 0.25 – 1.25 ng/ml.

Precision

Intra-assay coefficient of variation (n = 8)

serum	GP2a ng/ml	CV %
1	3.9	6.4
2	0.8	9.9
3	0.2	12.1

Inter- assay coefficient of variation (n = 8x5)

serum	GP2a ng/ml	CV %
1	3.9	7.8
2	0.8	10.3
3	0.2	29.5

Interference

GP2 containing sera were spiked with hemoglobin, triglycerides, bilirubin and GP2's urinary homolog Tamm-Horsfall protein (uromodulin). Final concentrations of 1.0 g/L hemoglobin, 30.0 mg/L bilirubin, 25.0 g/L triglycerides, and 10.0 g/L uromodulin did not interfere with the measurement of GP2a levels.

INCUBATION SCHEME

Pancreatitis GP2 (3950)

patients samples	Use undiluted sera

1	Bring all ready for use reagents to room temperature (18…25°C) before use.				
			calibrators	control	sera
2	Pipette	Calibrators (0 - 4) Controls (P) undiluted patient sera	100 µl	100 µl	100 µl
3	Incubate		60 minutes at room temperature		
4	Wash		Decant, Dispense 3 x 300 µl (made of B)		
5	Pipette conjugate (D)		100 µl	100 µl	100 µl
6	Incubate		30 minutes at room temperature		
7	Wash		Decant, Dispense 3 x 300 µl (made of B)		
8	Pipette substrate (E)		100 µl	100 µl	100 µl
9	Incubate protected from light		15 minutes at room temperature		
10	Pipette stop solution (F)		100 µl	100 µl	100 µl
11	Measure 450 nm versus 620 (690) nm				

SAFETY PRECAUTIONS

- **This kit is for in vitro use only**. Follow the working instructions carefully. GA GENERIC ASSAYS GmbH and its authorized distributors shall not be liable for damages indirectly or consequentially brought about by changing or modifying the procedure indicated. The kit should be performed by trained technical staff only.
- The expiration dates stated on the respective labels are to be observed. The same relates to the stability stated for reconstituted reagents.
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept at 2 - 8 °C before use in the original shipping container.
- Some of the reagents contain small amounts of Neolone M10 (< 0.1 % w/v) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed:
  - Do not smoke, eat or drink while handling kit material,
  - Always use protective gloves,
  - Never pipette material by mouth,
  - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.