DxPG80.Lab

IVD test for the quantitative measurement of hPG_{80} (circulating progastrin) in human EDTA plasma

Instructions for use

REF DxL2 (78 tests)
DxL4 (156 tests)

CE

UDI-DI: 03760314550048
UDI-DI: 03760314550055

IVD

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BIODENA CARE

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1. Intended Use

1.1 Intended purpose

IVD test for the quantitative measurement of hPG_{80} (circulating progastrin) in human EDTA plasma.
IVD: In vitro Diagnostics

1.2 Intended user

Staff of professional laboratories: trained technicians and biologists working in medical biology laboratory, certified ISO 15189 or equivalent.

1.3 Intended polulation

The DxPG80.Lab is intended for adults, male and female aged 18 years and older. The DxPG80.Lab is not intended for pregnant women or children.

2. Principle of the Test

The test is based on the principle of a sandwich ELISA to measure the concentration of hPG_{80} in plasma specimens that have been anticoagulated with EDTA. Briefly, a capture antibody specific for hPG_{80} is pre-coated on the 96-well microplate. hPG_{80} present in calibrators, controls and/or specimens added to the wells bind to the immobilized capture antibody. The DxPG80.Lab kit includes calibrators (CAL) which are used to estimate the level of hPG_{80} in EDTA plasma samples and controls (CTL). The wells are washed and an anti-hPG_{80} detection antibody coupled with horseradish peroxidase (HRP) is added, resulting in an antibody-antigen-antibody complex. After a second wash, a 3,3',5,5'-Tetramethylbenzidine (TMB) substrate solution is added to the well, producing a blue color in direct proportion to the amount of hPG_{80} present in the initial sample. The Stop Solution changes the color from blue to yellow, and the wells are read at 450 nm with a microplate reader.

3. Clinical background

Physiologically, progastrin, an 80 amino acid protein, is the precursor of the gastrointestinal hormone Gastrin, encoded by the GAST gene. It is synthetized by gastric antrum G cells, and then processed into Gastrin by multiple enzymatic processes [1]. In pathological conditions, the GAST gene was shown to be over-expressed in human colorectal tumor cells, leading to the accumulation of circulating progastrin (hPG_{80}) [2, 3]. As hPG_{80} is either unprocessed or poorly processed into Gastrin, it is released from the tumor cells and becomes detectable in the blood of colorectal cancer (CRC) patients [4, 5].

Initially believed to be biologically inactive, hPG_{80} was shown to participate in some features of a tumor, such as the disruption of cell-cell junctions [6], cell proliferation [7], inhibition of apoptosis [8], regulation of cancer stem cells [9], and angiogenesis [10]. In addition, targeting hPG_{80} with a specific antibody promotes apoptosis, decreases proliferation and migration/invasion of human colorectal cancer cells and inhibits self-renewal of colon cancer stem cells (CSC), as well as Wnt-driven tumorigenesis in mice [5]. Interestingly, GAST is a direct target of the Wnt/ß-catenin/Tcf4 oncogenic pathway [3] that is activated in many other cancers and plays a major function in cancer stem cells survival [11].

You et al., showed that hPG_{80} was present in the 11 tumor types tested [12]. hPG_{80} was detected in the blood of patients (n=1546) at significantly higher concentration than in healthy blood donors (n=557) with a median hPG_{80} of 4.88 pmol/L versus 1.05 pmol/L ($p<0.0001$), respectively. They also showed a correlation between *GAST* mRNA levels in the matched tumors and in the blood of the patients (i.e., lung cancers, Spearman r=0.8; p=0.0023). Furthermore, they showed a strong association between longitudinal hPG_{80} concentration changes and anti-cancer treatment efficacy in two independent retrospective studies. In the peritoneal carcinomatosis cohort, median hPG_{80} from inclusion to the post-operative period decreased from 5.36 to 3.00 pmol/L ($p<0.0001$, n=62) and in the hepatocellular carcinoma cohort, median hPG_{80} from inclusion to remission decreased from 11.54 pmol/L to 1.99 pmol/L ($p<0.0001$, n=63) [12]. More recently, other studies confirmed the presence of plasma hPG_{80} in of cancer patients [13, 14, 15].

4. Setup

The Instructions for Use must be read in its entirety prior to using this product.

- Unpack the components of the kit.
- Ensure that all accompanying components have been provided.
- Visually inspect the outer packaging. If any items are missing or if the packaging is damaged, do no use the kit and contact Support.

For In Vitro Diagnostic Use Only
For Professional Use Only

Users are expected to be proficient in the use of enzyme immunoassays and have a thorough understanding of the Instructions for Use prior to using this product. Users should follow good laboratory practices and comply with all training and proficiency requirements of the medical biology laboratory in accordance with local regulations. Failure to comply with these requirements and practices may lead to inaccurate results. Contact Support if you require additional training with the use of the test.

5. Warnings and Precautions

Labelling: Warning, GHS07 symbol
Contains biological material of human origin
Contains human blood or plasma derivatives

Classification according to Regulation (EC) No. 1272/2008:

- No hazards for components B30, R10 and R50.
- Component B41 is Skin Sensitisation – Category 1A
- Components R30, R60, CAL and CTL are Skin Sensitisation – Category 1
- Component R40 is Oral Acute Toxicity Category 4; Eye Irritation Category 2; Skin Irritation Category 2; Skin Sensitisation Category 1.
- Calibrators (CAL) and controls (CTL) contain a protein (hPG_{80}) of human origin. It is recommended to manipulate this ingredient as potentially hazardous.
- Component B70 is is not a hazardous substance or mixture according to Regulation (EC) No. 1272/2008 but contains human plasma. Although the human plasma was free of infectious agents at the time of its collection according to the European regulations (non-reactive for Hepatitis B Surface Antigen, HIV 1&2, and HCV antibodies), it is recommended to manipulate this ingredient as potentially hazardous and infectious.
- The human specimens required but not provided in this kit should be handled as potentially hazardous and infectious.

Hazard statements: Harmful if swallowed and when in contact with skin. Causes skin irritation, serious eye irritation and may cause an allergic skin reaction.

Precautions: Wear protective gloves, protective clothing and eye or face protection. Do not eat, drink or smoke when using this product. Avoid breathing mist or spray. Wash hands thoroughly after handling. Contaminated work clothing should not be allowed out of the workplace. Avoid release to the environment.

Response:

- If swallowed, rinse mouth and immediately call a poison center or a doctor if you feel unwell. Never give anything by mouth to an unconscious person. Do NOT induce vomiting.
- If inhaled, move into fresh air. If not breathing, give artificial respiration. Contact a poison control center or a doctor if you feel unwell.
- If in eyes, rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If eye irritation persists, get medical advice.
- If on skin, wash with plenty of soap and water. If skin irritation or rash occurs, get medical advice.
- Take off contaminated clothing and wash it before reuse.

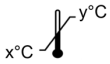
Disposal: Dispose of contents to a waste approved for Health or environmental hazard products, in accordance with national and/or international regulation.

For more information, consult the DxPG80.Lab Material Safety Data Sheets (MSDS), available upon request.

5.1 Symbols

The following symbols represent warnings/precautions and information for safety regarding the safe and effective use of the DxPG80.Lab test.

Table 1: Symbols

Symbol	Explanation	Symbol	Explanation	Symbol	Explanation
	Consult instructions for use		In vitro diagnostic (IVD) medical device		Do not use if package is damaged
	Caution		Manufacturer		Use-by date (YYYY-MM)
	Catalogue number		Batch code		Contains sufficient for <n> tests
	Positive control		Negative control		Do not re-use
	Store between x°C and y °C temperature		Keep dry		Contains biological material of human origin
	Warning		Danger		Contains human blood or plasma derivatives

5.2 Technical Precautions

All kit components have been formulated and tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance. Do not mix or substitute reagents or materials from other kit lots or manufacturers.

AVOID

- mixing the components/reagents of different lots of the kit. Discard all the reagents of the kit once the plates have been used.
- re-using 1X preparations. Discard all 1X preparations after use.
- freeze-thaw cycles of calibrators and controls. Discard after use.
- freeze-thaw cycles of the plasma samples, no more than three cycles.
- foaming or bubbles when mixing or reconstituting kit components.
- exposing the substrate solution to bright light or to oxidizing agents.
- cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- using the kit after the expiration date.

ENSURE

- that all necessary reagents and equipment ready on hand before starting. Once the assay has been started all steps should be completed with precise timing and without interruption.
- that all samples and buffers are mixed gently but thoroughly before use and that none of the chemicals have precipitated.
- that no reagent shows signs of bacterial growth, if so, discard the contaminated reagent.
- that all samples and reagents are added to the bottom of each well.
- that plates are covered during incubation steps.
- the complete removal of all solutions and buffers during wash steps to minimize background.
- that there are not any air bubbles in the well after the addition of the stop solution as bubbles interfere with spectrophotometric readings.
- that immediately after use reagents are returned to their proper storage conditions.
- the use of a pre-loading 96-well DeepWell plate to prepare samples and controls. It will allow an efficient control of the loading time duration among samples and controls and will avoid an increase in the background values.
- that kit reagents are regarded as hazardous waste and disposed of in accordance with local/regional and national regulations.

6. Limitations

- Any inappropriate handling of samples, modifications to this test and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.
- If specimens generate values higher than 50 pmol/L, dilute the specimens in hPG_{80} negative EDTA plasma (ref. B70) and repeat the test.
- If automated equipment is used with the test, a complete validation of the test using the automated equipment should be conducted to meet the performance characteristics specified in the Instructions for Use.
- The results obtained with this test should never be used as the sole basis for a clinical diagnosis and/or therapeutic consequences. The test results should be used by a physician in conjunction with information available from clinical evaluations and other diagnostic procedures.
- Each testing laboratory should establish its own normal reference range.
- Any off-label use of this test is the responsibility of the user; the manufacturer cannot be held liable.
- Report any serious incident that has occurred in relation to the device to the manufacturer and the competent authority of the member state in which the user and/or the patient is established.

7. Materials

7.1 Materials Provided

Table 2: DxPG80.Lab components

Ref.	Component	DxL2 units	DxL4 units	Use	Storage T°C	Storage opened
B30	hPG80 microplate, 96 wells	2	4	Ready to use	BOX 1	6 months – Store unused B30 wells with desiccant in the sealed foil pouch.
B41	10X Concentrated conjugate, 3 mL	1	2	Dilute see § 9	8°C 2°C	6 months
R10	20X Wash solution, 60 mL	1	2			
R30	Sample dilution buffer, 60 mL	1	1	Ready to use		
R40	Stop solution, 60 mL	1	1			
R50	Substrate solution, 60 mL	1	1			
R60	Conjugate dilution buffer, 60 mL	1	1			
B70	Negative EDTA plasma, 8 mL	1	2	Ready to use	BOX 2	Discard after 3 freeze-thaw cycles.
B93	120X CAL 0 pmol/L, 20 µL	1	2	Dilute see § 9	-70°C -90°C	Discard after use
B94	120X CAL 5 pmol/L, 20 µL	1	2			
B95	120X CAL 15 pmol/L, 20 µL	1	2			
B96	120X CAL 25 pmol/L, 20 µL	1	2			
B97	120X CAL 35 pmol/L, 20 µL	1	2			
B98	120X CAL 50 pmol/L, 20 µL	1	2			
C71	120X CTL 5 pmol/L, 20 µL	1	2	Dilute see § 9		
C72	120X CTL 25 pmol/L, 20 µL	1	2			
C73	120X CTL 40 pmol/L, 20 µL	1	2			

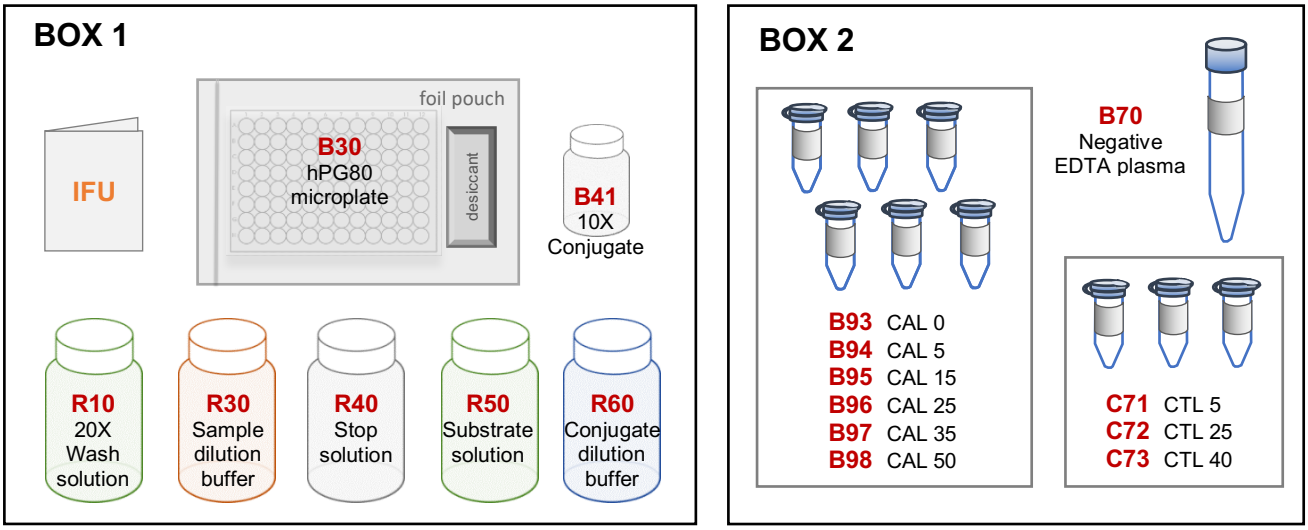


Figure 1: DxPG80.Lab layout of box 1 and box 2

7.2 Materials not supplied

Required for blood collection at the the pre-analytical lab:

- Blood-collecting materials (syringe, etc.),
- Enough K2 or K3 EDTA tubes to hold 8 mL of whole blood (such as Vacutainer® K2-EDTA BD #367525 or equivalent),
- Labels for patient identification traceability.

Required for plasma processing at the the pre-analytical lab:

- 4°C refrigerated centrifuge 1,300 g to 2,000 g force, bucket compatible with the size of the EDTA collection tubes used,
- If plasma is stored frozen, freezer-resistant labels and 3 mL cryotubes (such as Clearline Cryotubes 3 mL Dutscher #390703 or Cryogenic Vials, External Thread 3 mL VWR #10018-762 or equivalent).

Required for storage and shipping:

- Refrigerator +2°C to +8°C (immediate storage of blood and plasma),
- Freezer -20°C (short-term storage of plasma) or -80°C (long-term storage of plasma).

Check that the carrier is authorized to ship biological substances category B, code 3373.

Required for testing at the analytical lab:

- 96-well microplate reader capable of measuring absorbance at 450 nm
- 96-well DeepWell Polypropylene Microplates to use for the pre-load plate
- Multi-channel calibrated pipettes delivering volumes of 50-300 µL and dispensing reservoir
- Single-channel calibrated pipettes delivering volumes of 2-1000 µL and disposable tips
- Serological 1 mL, 5 mL, 10 mL, 25 mL pipettes for reagent preparation and pipetboy
- Polypropylene Microtubes with lid, 1.5 mL and microtube rack
- Distilled or Deionized water
- Manual equipment for dispensing wash solution in wells (i.e. multichannel pipette, manual washer manifold) or automated microplate washer
- Vortex (and optional: small tabletop centrifuge for short spin of 1.5 mL tubes)
- Laboratory timer
- Absorbent paper
- Aluminium foil to store the 1X conjugate in the dark
- Lid or paraffin film to cover microplate during incubation(s)
- Adhesive tape to securely attach strips to the microplate frame during washing steps
- Laboratory oven allowing a stable temperature of 37°C ± 2°C
- Cold +2°C to +8°C handling device (ice, cold tube rack or refrigerated plate holder)
- 1000 mL graduated cylinder
- 1 L glass bottle for wash preparation

8. Specimens

8.1 Specimen collection

1. Collect 8 mL of whole blood in K2-EDTA or K3-EDTA tubes, as per tube supplier recommendations, by venipuncture from adult patients (see §1.3 population) who present a medical prescription for the hPG_{80} test. For traceability purposes, the tube labels and medical prescription must present patient identification (last name, first name, date of birth, identification code…).
2. Immediately invert 10 times the blood collection tubes to mix blood and anticoagulant.

8.2 Plasma recovery

1. Centrifuge the blood collection tubes for 10 minutes at +4°C at 1,300 g (or between 1,300 g and 2,000 g) using a refrigerated centrifuge to remove the cells. For g to rpm conversion, refer to the centrifuge rotor specification. A break can be used to stop the centrifuge faster.
2. Collect plasma following centrifugation, ideally, 4 mL / patient. It is important to immediately and carefully transfer 2 mL plasma into a cryotube dedicated to shipping. Keep the rest (about 2 mL plasma) in a second cryotube for backup. Ensure specimen traceability with at minimum a unique identifier.
3. Maintain plasma between +2°C and +8°C. Inspect for color and turbidity: plasma should be deemed clear (not hemolyzed and non-lipemic) by an experienced technician. Turbid plasma should be centrifuged and aspirated again to remove remaining insoluble matter.

8.3 Specimen storage

1. If the whole blood cannot be centrifuged immediately, store the blood collection tubes between +2°C and +8°C, protected from light, and if possible in a vertical position, for a maximum of 48 hours (2 days) including no more than 4 hours at room temperature (between about +15°C and +25°C). Do not freeze whole blood.
2. If the plasma cannot be tested for hPG_{80} immediately, store the plasma tubes between +2°C and +8°C for a maximum of 2 hours or at -20°C ± 5°C for a maximum of three (3) months.

Transfer the plasma tubes to -80°C ± 10°C for long term storage. No more than three freeze-thaw cycles of the plasma samples are allowed, storage and handling included.

3. Upon receiving DxPG80.Lab test results, any remaining tube may be discarded as per local regulation.

8.4 Specimen shipping

Refer to transport regulation on biological substances category B, code 3373.

- Whole blood is shipped between +2°C and +8°C for a maximum of 48 hours (2 days).
- Plasma should be delivered frozen.

8.5 Transmission of patient data

Patient data must be transmitted to the laboratory receiving the specimen when specimens are shipped. The last name and first name should be noted separately in the patient identification and associated with an identification code, a date of birth and when possible, the gender.
The name of the person who will receive test results should also be stated. Note any deviation and inform the analytical laboratory receiving the specimen and your Biodena Care contact.

9. Assay Preparation

Ensure that an incubator is at +37ºC prior to starting the procedure. Bring all reagents to room temperature (between +15 and +25°C) prior to use, except 10X Concentrated conjugate, CAL, CTL and Negative EDTA plasma which should be kept between 2°C and 8°C prior to use. **Ensure that all reagents are homogenous by inversion or vortex and a quick spin. Do not use reagents that have precipitates.**

1X Wash solution

1. Bring the 20X Wash solution (ref. R10) to room temperature (between about +15°C and +25°C) and mix thoroughly to ensure that the Wash solution is completely solubilized.

2. Before performing the dilution, visually inspect the 20X Wash solution (ref. R10). If the presence of crystals is detected, dissolve the salt crystals by mixing and careful warming until the crystals have disappeared. Don't warm above 37°C.

3. Dilute 1 → 20 µL the 20X Wash solution in distilled or deionized water, for example:
   - ready to use for 1 plate: 25 mL 20X Wash + 475 mL water = 500 mL 1X Wash
   - ready to use for 2 plates: 50 mL 20X Wash + 950 mL water = 1000 mL 1X Wash.

Calibrators CAL and Controls CTL

The kit includes 6 calibrators, CAL 0 to 50 pmol/L, (ref. B93 to B98) and 3 controls, CTL 5 to 40 pmol/L, (ref. C71, C72 and C73), that are supplied concentrated 120X and that must be diluted to 1X in Negative EDTA plasma (ref. B70) as described below.

1. Thaw 120X CAL and CTL between +2 and +8°C. Vortex. Once thawed **CAL and CTL must be handled between +2 and +8°C** (over ice or cold rack) during the preparation. Blue color may appear in thawed CAL and CTL but does not impact results.

2. Thaw Negative EDTA plasma (ref. B70) at room temperature (on the bench or in a water bath at room temperature). Vortex. No more than **three (3) freeze-thaw cycles** of the Negative EDTA plasma are allowed. Storage of B70 at -20°C ±5°C is acceptable up to 3 months only.

3. Dilute 1 → 120 µL the 120X CAL or CTL in Negative EDTA plasma (ref. B70) for example:
   - ready to use for 1 or 2 plates: *2 µL 120X CAL + 238 µL B70 = 240 µL 1X CAL
   - ready to use for 3 plates: 3 µL 120X CAL + 357 µL B70 = 360 µL 1X CAL
   - ready to use for 4 plates: 4 µL 120X CAL + 476 µL B70 = 480 µL 1X CAL.

   *Note : Do not pipette less than 2 µL, which reduces precision.

4. Vortex briefly to ensure the homogeneity of the calibrators and control solutions. Optional: After vortex, droplets in lid may be removed by a flick or quick spin if necessary.

1X Conjugate

Fifteen (15) minutes before the end of the first incubation step (see section 10.4 or 11.3), dilute 1 → 10 µL the 10X Concentrated conjugate (ref. B41) in Conjugate dilution buffer (ref. R60) for example:

- ready to use for 1 plate: 200 µL 10X Conjugate + 1.8 mL buffer R60 = 2 mL 1X Conjugate
- ready to use for 2 plates: 400 µL 10X Conjugate + 3.6 mL buffer R60 = 4 mL 1X Conjugate

Keep the 1X conjugate freshly prepared in the dark using aluminuim foil around the tube.

hPG80 microplate

The hPG80 microplate (ref. B30) is composed of 12 removable strips (8 wells/strip) that are ready to use. It is not necessary to rinse the plate prior to adding reagents. Each plate can accomodate 39 samples and should contain duplicates of each CAL and CTL, see example of plate design below. Use the number of strips needed for the assay.

1. Remove excess strips from the plate frame, return them to the foil pouch with the dessicant. Seal pouch. Once opened, the hPG80 microplate strips can be stored between +2°C and +8°C for up to 6 (six) months.
2. Before proceeding to the assay procedure, carefully identify every strip with a permanent pen.
3. Attach securely the strips to the frame using adhesive tape so strips do not fall off the frame during washing steps. Remove adhesive tape before OD readings.
4. After OD readings, discard used strips but keep frame.

Pre-load plate

Samples, CAL and CTL must be tested in duplicate and handled between 2°C and 8°C (over ice or refrigerated plate).

1. Transfer the prepared 1X CAL, 1X CTL and 130 µL of each sample in the 96-Well DeepWell Polypropylene Microplate (Pre-load plate), see an example of pre-plate design in Figure 3. Each well will be used to dispense a duplicate of each CAL, CTL and sample in a timely manner.
2. Cover the plate with a lid or paraffin film before use and keep it between +2 and +8°C.

	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL 50	CTL 5	8	16	24	32						
B	CAL 35	1	9	17	25	33						
C	CAL 25	2	10	18	26	34						
D	CAL 15	3	11	19	27	35						
E	CAL 5	4	12	20	28	36						
F	CAL 0	5	13	21	29	37						
G	CTL 40	6	14	22	30	38						
H	CTL 25	7	15	23	31	39						

Figure 2: Example of pre-load plate design, CAL: calibrator, CTL: control, 1-39: patient plasma samples

10. Assay Procedure

	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL 50	CAL 50	CTL 5	CTL 5	8	8	16	16	24	24	32	32
B	CAL 35	CAL 35	1	1	9	9	17	17	25	25	33	33
C	CAL 25	CAL 25	2	2	10	10	18	18	26	26	34	34
D	CAL 15	CAL 15	3	3	11	11	19	19	27	27	35	35
E	CAL 5	CAL 5	4	4	12	12	20	20	28	28	36	36
F	CAL 0	CAL 0	5	5	13	13	21	21	29	29	37	37
G	CTL 40	CTL 40	6	6	14	14	22	22	30	30	38	38
H	CTL 25	CTL 25	7	7	15	15	23	23	31	31	39	39

Figure 3: Example of plate design, CAL: calibrator, CTL: control, 1-39: patient plasma samples

1. Prepare all reagents, controls, and samples as directed in the previous section, except the 1X Conjugate.
2. Add using reverse pipetting 50 µL of Sample Dilution Buffer (ref. R30) per well of the hPG80 microplate (ref. B30) at room temperature. Reverse pipetting avoids the creation of air bubbles.
3. Add 50 µL of the 1X CAL, 1X CTL and samples with a multi-channel pipette (8 channels) from the Pre-load plate to the hPG80 microplate (ref. B30) at room temperature. Pre-wet the tips with two ups and downs in the preparation plate, then load samples by normal pipetting. The loading time should not exceed 10 minutes.
4. Cover the hPG80 plate with a paraffin film. Incubate for **1 hour** ± 5 min at **+37°C** ± 2°C.
5. Prepare the 1X Conjugate (ref. B41) as described in section 9.
6. At the end of the incubation step, discard all the liquid from the wells by inverting the plate. Proceed to a thorough washing step by adding 250 µL per well of **1X Wash solution** using a multi-channel pipette. Discard the 1X Wash solution by inverting the plate and thoroughly pat dry the plate frame upside down on absorbent paper. Repeat the washing step 6 times. At the end of the washing steps, ensure the complete removal of the liquid from the wells: all liquid has been successfully removed when no sign of liquid remains on the paper towel.

   Caution: The wash procedure is critical. Insufficient washing may result in poor precision and falsely elevated absorbance readings.
7. Add 100 µL of the **1X Conjugate** to each well using a multi-channel pipette. **This step should be done by reverse pipetting to avoid the creation of air bubbles**.
8. Cover the plate with a paraffin film. Incubate for **30 min** ± 3 min at **+37°C** ± 2°C.
9. Proceed to washing as described in step 6.
10. Add 100 µL of the **Substrate solution** (ref. R50) to each well using a multi-channel pipette. **This step should be done by reverse pipetting to avoid the creation of air bubbles.**
11. Incubate **in the dark** for **15 min** at **+37°C** ± 2°C.
12. Without removing the content of the wells, add 100 µL of the **Stop solution** (ref. R40) to each well using a multi-channel pipette in order to stop the reaction. **This step should be done by reverse pipetting to avoid the creation of air bubbles.**
13. Remove the tape from strips before OD readings. Read and record the OD at 450 within 5 minutes of adding the R40. Keep plate frame for unused strips.

11. Assay Procedure Summary

1. Prepare all reageants and plates as stated in section 9, except Conjugate.

↓

2. Add 50 µL Sample dilution buffer (ref. R30) per well of the hPG80 microplate (ref. B30)

↓

3. Transfer 50 µL per well of CAL, CTL and sample from the Pre-load plate in **less than 10 min**.
Incubate for 1 h at 37°C ± 2°C

↓ Prepare 1X Conjugate during this incubation

4. Discard the liquid by inverting the plate
Wash 6 times with 250 µL of 1X Wash solution 

↓

5. Add 100 µL 1X Conjugate
Incubate for 30 minutes at 37°C ± 2°C

↓

6. Discard the liquid by inverting the plate
Wash 6 times with 250 µL of 1X Wash solution 

↓

7. Add 100 µL Substrate solution (ref. R50)
Incubate for 15 minutes at 37°C ± 2 °C

↓

8. Add 100 µL Stop solution (ref. R40)

↓

9. Read and record the 450 nm OD

The wash procedure is critical, insufficient washing may result in poor precision and falsely elevated absorbance readings. Wash inverting the plate and thoroughly pat dry the plate upside down on absorbent paper. After 6 washes, make sure the liquid has been removed, the last paper must stay dry.

12. Data Analysis

The test is accepted when the acceptance criteria below have been met. When at least one criterion is “Rejected”, the test should be performed again. Please contact Support for repetitive rejected tests.

12.1 Standard Curve Calculation

A standard curve should be generated for each set of specimens assayed.

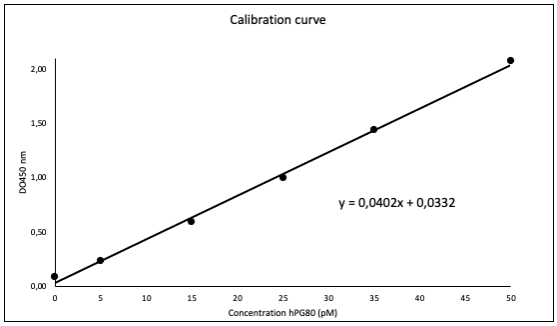
1. Calculate the mean OD values obtained from each CAL.
2. Report each of the 6 CAL point on a graph, where y-axis presents the mean ODs and x-axis presents the hPG_{80} concentrations in pmol/L.
3. Use CAL 0 as the anchor point.
4. Calculate the linear regression **y = ax + b**, where **a** is the regression slope and **b** is the OD intercept.

The regression slope should be significant:

Slope acceptance criteria	a ≥ 0.030

Example of a typical standard curve: these data are for demonstration only.

CAL (pmol/L hPG_{80})	Mean OD (450 nm)
CAL 0	0.09
CAL 5	0.23
CAL 15	0.59
CAL 25	1.00
CAL 35	1.44
CAL 50	2.07



12.2 Calibrator Acceptance Criteria

Calculate hPG_{80} concentrations in each calibrator CAL using the linear regression. Each calibrator is acceptable if the calculated value falls within or equal to the range indicated in the table below.

$$C = (\text{mean } OD_{CAL} - \text{OD intercept}) / \text{slope}$$

CAL	Acceptable range of hPG_{80} concentration (pmol/L)	% CV accepted
5	3.8 – 6.3	25
15	12 – 18	20
25	20 – 30	
35	28 – 42	
50	40 – 60	

12.3 Negative Control Acceptance Criteria

The assay negative control CAL 0 is acceptable if the mean OD falls within or equal to the range below.

CAL	Acceptable range of mean OD (450 nm)	% CV accepted
0	≤ 0.19	20

12.4 Positive Control Acceptance Criteria

Calculate hPG_{80} concentrations in each control CTL using the linear regression. Each control is acceptable if the calculated value falls within or equal to the range indicated in the table below. The plate results are acceptable if all three controls are accepted.

$$C = (\text{mean } OD_{CTL} - \text{OD intercept}) / \text{slope}$$

CTL	Acceptable range of calculated hPG_{80} concentration (pmol/L)	% CV accepted
5	3.8 – 6.3	25
25	20 – 30	20
40	32 – 48	

12.5 Calculation of plasma sample hPG_{80} concentration

Calculate hPG_{80} concentration in each sample using the linear regression.

$$C = (\text{mean } OD_{SAMPLE} - \text{OD intercept}) / \text{slope}$$

13. Test Results

Table 3: DxPG80.Lab result according to the levels of hPG_{80}

hPG_{80} level	Interpretation
Result < 1 pmol/L	Not detected
1 pmol/L ≤ Result < 3.3 pmol/L	< 3.3 pmol/L
3.3 pmol/L ≤ Result ≤ 50 pmol/L	____ pmol/L
Result > 50 pmol/L	____ pmol/L [1]

1. The concentration of hPG_{80} was beyond the range of the assay. Result is obtained after sample dilution in Negative EDTA plasma (ref. B70) and retest.

14. Analytical Performance

14.1 Limit of Detection and Quantitation

Measuring intervals	hPG_{80} (pmol/L)
LoD: Limit of Detection	1.00
LLoQ: Lower Limit of Quantitation	3.30
ULoQ: Upper Limit of Quantitation	50
Linear range	0 - 70

In theory, sandwich ELISAs with washing steps are not subject to a hook effect. This was confirmed for DxPG80.Lab test with measurement done between 0 and 250 pmol/L hPG_{80}.

14.2 Analytical Specificity

Cross-Reactant	Final concentration	Recovery (%)
Keyhole limpet hemocyanin	2 µg/mL	103 – 120
Carcinoembryonic antigen	20 µg/mL	94 – 103
Prostate specific antigen	210 mg/mL	95 – 103
Cancer antigen 125 (CA 125)	2000 U/mL	101 – 106
Cancer antigen 15-3 (CA 15-3)	100 U/mL	103 – 104
Gastrin-17 (G17)	2 µg/mL	90 – 104
G-Gly	2 µg/mL	101 – 106

Cross-reactivity was assessed using human plasma samples spiked with native hPG_{80} concentrations ranging from negative to strong positive and a fixed concentration of each potential cross-reactants. No cross-reactivity was detected with DxPG80.Lab.

Potentially interfering substances	Final concentration	Recovery (%)
Triglycerides	0.05 mg/mL	107 – 117
Cholesterol	25 µg/mL	96 – 104
Hemoglobin	2 mg/mL	101 – 111
Conjugated Bilirubin	0.5 µg/mL	94 – 101
SN-38	60 µM	94 – 105
5-FU	3 mM	96 – 122

Interference was also assessed using human plasma samples spiked with native hPG_{80} concentrations ranging from negative to strong positive and the concentration of each potentially interfering substances shown below. To determine if the added substance interfered with the assay performance, the recovery of hPG_{80} concentrations in each specimen was calculated. No interference was detected.

14.3 Accuracy

Accuracy was assessed by determining the recovery of three hPG_{80} concentrations (CTL 5, 25 and 40 pmol/L) in negative EDTA plasma with their nominal values. The highest mean deviation between the nominal value and the calculated concentration of hPG_{80} solution was 9.7 %.

CTL	Accuracy	Acceptance limit
5	5.8 %	25 %
25	9.0 %	20 %
40	9.7 %	20 %

14.4 Precision

The coefficients of variation (CV%) were determined using three CTL: 5, 25 and 40 pmol/L.

CTL	Inter-run precision	Acceptance limit
5	14.0 %	25 %
25	6.9 %	20 %
40	8.1 %	20 %

14.5 Total Error

The highest total error of hPG_{80} quantitation is 32.0% obtained for the CTL near the LLoQ.

CTL	Bias	SD WL	Total error	Acceptance limit
5	0.29	0.66	32.0 %	40 %
25	2.26	1.61	21.7 %	30 %
40	3.87	2.92	24.0 %	30 %

Total Error is defined as follows by the CLSI EP21 Evaluation of Total Analytical Error for Quantitative Medical Laboratory Measurement Procedures:

$$TAE = |Bias| + t \bullet SD_{WL}$$

where TAE is the Total Analysis Error, Biais is the inter-run accuracy, t is 1.96 for a 95% two-sided confidence and SDWL the inter-run precision.

15. Clinical Reference Range

The normal level of hPG_{80} in a control population was measured in n=1199 healthy volunteers with a balanced number of each gender, median age 51 years old (18 to 86 years old). Levels of hPG_{80} were also measured in n=3507 cancer patients, median age 65 years old, from a cohort including several cancer types: breast, prostate, colorectal, liver, gynecologic (ovarian, endometrium and uterus), kidney, esophagus/stomach, skin melanoma, neuroendocrine, lung, pancreas, brain (glioblastoma and glioma), head and neck, uveal melanoma, sarcoma, lymphoma, bladder, thyroid and leukemia.

Table 4: Clinical ranges for hPG_{80}

hPG_{80} levels	Controls	Cancers
Reference range, pmol/L [for 95% of the control population]	0 – 10.9	n/a
Interquartile range, pmol/L [for 75% of the population]	0.3 – 3.5	1.5 - 10.5
Median, pmol/L	1.5	4.0
Mean (standard error), pmol/L	4.2 (15.2)	13.2 (40.1)
Number of samples	1199	3507

In the case of hPG_{80}, only high values could be of clinical concern, and therefore the 5% of outlying observations for the reference interval are identified only at the high end of the distribution of

asymptomatic donor values, resulting in a one-sided reference interval. Based on our current data available, please note that 20% of cancer patients do not have detectable hPG_{80} in the blood plasma.

Each laboratory should determine its own normal values.

DxPG80.Lab is not a stand-alone test intended for cancer diagnostics or treatment monitoring, all results must be interpretated by the prescribing physician in conjunction with information available from clinical evaluation and other diagnostic procedures.

16. References

1. Rehfeld JF, Zhu X, Norrbom C et al. Prohormone convertases 1/3 and 2 together orchestrate the site-specific cleavages of progastrin to release gastrin-34 and gastrin-17. Biochem J 2008; 415: 35-43.
2. Van Solinge WW, Nielsen FC, Friis-Hansen L et al. Expression but incomplete maturation of progastrin in colorectal carcinomas. Gastroenterology 1993; 104: 1099-1107.
3. Koh TJ, Chen D. Gastrin as a growth factor in the gastrointestinal tract. Regul Pept 2000; 93: 37-44.
4. Siddheshwar RK, Gray JC, Kelly SB. Plasma levels of progastrin but not amidated gastrin or glycine extended gastrin are elevated in patients with colorectal carcinoma. Gut 2001; 48: 47-52.
5. Prieur A, Cappellini M, Habif G et al. Targeting the Wnt Pathway and Cancer Stem Cells with Anti-progastrin Humanized Antibodies as a Potential Treatment for K-RAS-Mutated Colorectal Cancer. Clin Cancer Res 2017; 23: 5267-5280.
6. Hollande F, Lee DJ, Choquet A et al. Adherens junctions and tight junctions are regulated via different pathways by progastrin in epithelial cells. In J Cell Sci. 2003; 1187-1197.
7. Singh P, Owlia A, Varro A et al. Gastrin gene expression is required for the proliferation and tumorigenicity of human colon cancer cells. Cancer Res 1996; 56: 4111-4115.
8. Wu H, Owlia A, Singh P. Precursor peptide progastrin1–80 reduces apoptosis of intestinal epithelial cells and upregulates cytochrome c oxidase Vb levels and synthesis of ATP. In Am J Physiol Gastrointest Liver Physiol. 2003; 1-14.
9. Giraud J, Failla LM, Pascussi JM et al. Autocrine Secretion of Progastrin Promotes the Survival and Self-Renewal of Colon Cancer Stem-like Cells. In Cancer Res. 2016; 3618-3628.
10. Najib S, Kowalski-Chauvel A, Do C et al. Progastrin a new pro-angiogenic factor in colorectal cancer. Oncogene 2015; 34: 3120-3130.
11. Katoh M, Katoh M. Molecular genetics and targeted therapy of WNT-related human diseases (Review). Int J Mol Med 2017; 40: 587-606.
12. You B, Mercier F, Assenat E et al. The oncogenic and druggable hPG80 (Progastrin) is overexpressed in multiple cancers and detected in the blood of patients. EBioMedicine 2019; 51: 102574.
13. Kohli M. TanW. Vire B. Liaud P. Blairvacq M. Berthier F. Rouison D. Garnier G. Payen L. Cousin T. et al. Prognostic Value of Plasma hPG80 (Circulating Progastrin) in Metastatic Renal Cell Carcinoma. Cancers 2021 13 375.
14. Dupuy M. Iltache S. Rivière B. Prieur A. Pageaux G.P. Bedoya J.U. Faure S. Guillaumée H. Assenat E. Plasma hPG80 (Circulating Progastrin) as a Novel Prognostic Biomarker for Hepatocellular carcinoma. Cancers 2022 14 402.
15. Chauhan A, Prieur A, Kolesar J, Arnold S, Payen L, Mahi Y, Vire B, Sands M, Evers B.M., Joubert D et al. hPG80 (Circulating Progastrin), a Novel Blood-Based Biomarker for Detection of Poorly Differentiated Neuroendocrine Carcinoma and Well Differentiated Neuroendocrine Tumors. Cancers 2022, 14, 863.
16. Cappellini M, Flaceliere M, Saywell V, Soule J, Blanc E, Belouin F, Ortiz E, Canterel-Thouennon L, Poupeau S, Tigrett S, Vire B, Liaud P, Blairvacq M, Joubert D, Prieur A. A novel method to detect hPG80 (human circulating progastrin) in the blood. Anal Methods. 2021 Oct 8;13(38):4468-4477. doi: 10.1039/d1ay00986a. PMID: 34494619.

Revision History

Rev	Date	Summary of Change
1	2021.07.30	Creation of document based on DxPG80-IFU-01.r9 with the following modifications: CAPA-2021-03: Adult patients (18 years or more) modified in sections 1 and 8.1 CC-033A: stability extension modified in section 7.1. CC-042: Creation of DxPG80.Lab (ref. DxL) based on DxPG80 (ref. Dx), all sections concerned, new logo. CC-043: Extension of the calibration range (from 25 to 50 pmol/L), all sections concerned. CC-048: blood storage allowed up to 48h in section 8. CC-050: Clinical interpretation updated in section 13. CC051: IVDR update, add UDI-DI in header with number of tests per DxL2, DxL4. And minor changes: Section 3. Clarification between physiological Progastrin and oncogenic hPG80. Section 7.1 add layout and unopened storage. Section 10.9 Plate example starting with CAL50. Section 12.2 Update the calibration curve.
2	2021.11.04	DXL-PDR.r1 requirement 10.2.43: add bacteria check (§5.2). CC-047: add slope criteria (§12.1) change CAL 0 ≤ 0.19 (§12.4). Minor changes: add LOD-LOQ table and correct hook effect up to 250 pmol/L (§14.1).
3	2022.02.17	CC-046: add specimen instructions and materials from DXL-WIN-011 due to cancelled CKC-CKB, in §7.2 and 8. CC-061: MSDS update (§5). CC-053: Extend expiry date after opening for R30, R40, R50 and R60 in §7.1 CC-063: add Biodena Care logo in footer Minor correction (typo) 12-18 pM range for CAL 15 in §12.3 and FC-2020-01: blue color of CAL and CTL in 9.1. Update of mean OD and curve in §12.2, update of data in §14.3 Accuracy, §14.4 Precision and §14.5 Total error.
4	2022.07.27	CC-055: Plasma sample can be stored at -20°C for 3 months instead of 1 month (§8.3.2). CC-070: Wash volume is reduced from 300 to 250 µL per well (§10.6, 11.4 and 11.6). CC-072: Total blood volume is lowered from 10 to 8 mL (§7.2 and 8.1.1) and plasma volumes are adjusted accordingly (§8.2.2). Minor change: remove carefully (§8.1.2). FC-2022-03: add 'if possible' for vertical storage (§8.3.1). Remove (01) before UDI-DI (front page).
5	2022.10.24	CC-073: Remove the requirement on the fasting state of the patient (§1.3 and 8.1). CC-077: Extend to 6 months the open expiry date of box 1 components in table 2 and §9.hPG80.1. CC-079: Possibility to store B70 plasma for maximum 3 months at -20°C in §9. CC-081: Update result interpretation in table 3. Update Control range and delete cancer range in §13 and 15. Add 3 publications to §3 and §16. Minor changes: update logo color; add foil and mandatory scotch in §7.2 and §9.conjugate; add calculations in §9; add frame and Pre-load design in §9.hPG80.4; remove tape and read within 5 min in §10.13; group §12.1 and 12.2 curve calculations and example; include all data from §14.1 in a table.
6	2022.10.25	CC-064: Add bag in table 2 and figure 1. Change incubation temperature from 37°C to 30°C (§7.2, 9, 10.4, 10.8, 10.11 and 11) to suit REACH-driven minor composition change of R30. Change applicable to lot 0006 and following lots of DxPG80.Lab. [IFU.r6 not distributed]
7	2023.01.04	FC-2022-13: based on IFU.r5 with reference range kept in §15 but removed from §13, section renamed Test result. More explicit hook effect in §14.1. Cancer data added in §15.

Approvals

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