



## 1. NAME AND INTENDED USE

GASK-PR is a radioimmunoassay kit for the direct quantitative determination of gastrin in human serum or plasma.

## 2. INTRODUCTION

Gastrin is a gastro-intestinal hormone secreted by the G cells of the gastric antral mucosa and by the duodenum. There are several forms of gastrin in the blood: big big gastrin, big gastrin (G 34), little gastrin (G 17) and mini gastrin (G 14). The biological activity common to all these forms is bound to the C-terminal tetrapeptide. Plasmatic gastrin has a half-life of approximately 8 minutes, and its metabolism is mainly renal.

The main factors controlling gastrin secretion are antra mechanical distension, alimentary contact, central stimulation via the pneumogastric nerve, gastrointestinal hormones and medicines. Antral pH is the main regulator of gastrin secretion, as it is suppressed when the pH descends below 3.

Gastrin stimulates not only the secretion of gastric acid, but that of intrinsic factor, of bicarbonate secretion by the pancreas, and the release of insulin and calcitonin. Indirectly, it stimulates pepsin secretion. It also has a trophic effect on the digestive mucosa and plays a role in digestive motricity.

The main application of gastrin assays lies in the early diagnosis of Zollinger-Ellison syndrome, a gastrin-secreting tumor usually of pancreatic origin. Such cases give a clear-cut gastrin reaction to the secretin test.

High basal gastrin levels have been reported in cases of antral G cell hyperplasia, of atrophic gastritis, of Biermer's anemia and of vagotomy.

Correct interpretation of gastrinemia requires a knowledge of the patient's basal and post-stimulation gastric acid secretion, and of his or her renal function and age, as well as of the conditions under which samples were taken.

## 3. PRINCIPLE

The principle of the assay is based on competition between gastrin radiolabeled with iodine 125 and gastrin contained in the standards or samples to be assayed for a given limited number of anti-gastrin antibody sites.

At the end of the incubation period, the amount of radiolabeled gastrin bound to the antibody is inversely proportional to the amount of non-radiolabeled gastrin originally present in the assay.

The method proposed for the separation of the free and bound fractions uses an immunoprecipitant in which a second antibody has been precipitated in excess.

## 4. REAGENTS

Each kit contains enough reagents for 100 tubes. The expiry date is marked on the external label.

REAGENTS	QUANTITY	STORAGE
<b>125I GASTRIN:</b> lyophilized. Synthetic gastrin 1 (1-17), buffer, bovine albumin, red dye. $\leq 37$ kBq ( $\leq 1$ $\mu$ Ci). Reconstitute with 10 ml of distilled water.	1 qs 10 ml vial	2-8 °C until the expiry date. Use immediately after reconstitution: do not use beyond.
<b>ANTISERUM:</b> lyophilized. Anti-gastrin rabbit antiserum, buffer, normal rabbit serum, bovine albumin, preservative, blue dye. Reconstitute with 30 ml of distilled water.	1 qs 30 ml vial	2-8 °C until the expiry date. Use immediately after reconstitution: do not use beyond.
<b>STANDARDS:</b> lyophilized. Synthetic gastrin 1 (1-17), buffer, bovine albumin, sodium azide. 20 - 60 - 150 - 500 - 1500 $\mu$ U/ml*. Reconstitute with 1 ml of distilled water.	5 qs 1 ml vials	2-8 °C until the expiry date. Use immediately after reconstitution: do not use beyond.
<b>0 STANDARD:</b> lyophilized. Buffer, bovine albumin, sodium azide. Reconstitute with 5 ml of distilled water.	1 qs 5 ml vial	2-8 °C until the expiry date. Use immediately after reconstitution: do not use beyond.
<b>IMMUNOPRECIPITATING REAGENT:</b> ready for use. Insoluble complex of anti-rabbit IgG sheep serum and non-immunized rabbit IgG, buffer, polyethylene glycol, sodium azide.	1 100 ml vial	2-8 °C until the expiry date.

(\*) The values shown above are only target values: the true value of each standard is shown on its label. 1  $\mu$ U MRC 68/439 = 1.05 pg MRC 68/439.

## 5. PRECAUTIONS FOR USE

### 5.1. Safety measures

Raw materials of human origin contained in the reagents of this kit have been tested with licensed kits and found negative for the anti-HIV 1, anti-HIV 2, anti-HCV antibodies and the HBs antigen. However as it is impossible to strictly guarantee that such products will not transmit hepatitis, the HIV virus, or any other viral infection, all raw materials of human origin including the samples to be assayed must be treated as potentially infectious.

Do not pipette by mouth. Do not smoke, eat or drink in areas in which specimens or kit reagents are handled. Wear disposable gloves while handling kit reagents or specimens and wash hands thoroughly afterwards. Avoid splashing.

Decontaminate and dispose of specimens and all potentially contaminated materials as if they contained infectious agents. The recommended method for doing this is autoclaving for a minimum of one hour at 121.5°C.

Sodium azide may react with lead or copper piping to form highly explosive metal azides. During waste disposal, flush the drains thoroughly to prevent a build-up of these products.



## 5.2. Basic radioprotection rules

This radioactive product may only be received, purchased, stored or used by persons so authorized, and by laboratories covered by such authorization. The solution should under no circumstances be administered to humans or to animals.

The purchase, storage, use or exchange of radioactive products are subject to the laws in force in the user's country.

Enforcement of the basic radioprotection rules will ensure adequate security.

A summary of these is given below:

Radioactive products must be stored in their original containers in a suitable area. A record of the reception and storage of radioactive products must be kept up to date. Handling of radioactive products should take place in a suitably-equipped area with restricted access (controlled zone). Do not eat, drink, smoke or apply cosmetics in a controlled zone. Do not mouth-pipette radioactive solutions. Avoid any direct contact with all radioactive products by using laboratory coats and protective gloves. Contaminated laboratory equipment and glassware must be disposed of immediately after contamination to prevent cross-contamination of different isotopes. Any contamination or radioactive substance loss should be dealt with in accordance with the established procedures. All radioactive waste disposal must be carried out according to the regulations in force.

## 5.3. Handling precautions

Do not use kit components beyond their expiry date. Do not mix reagents from different batches. Avoid any microbial contamination of reagents or of the water used for the reconstitution of reagents. Before each incubation, well mix the content of the tubes to ensure an homogeneous medium. Respect the recommended speed and length of centrifuging time in order to ensure the adherence of the immunoprecipitate to the bottom of the tubes. The use of trays enabling the simultaneous turning over of several tubes is recommended. Draining on an absorbent support is necessary to obtain reproducible results (better duplicates, reduced non specific activity). Do not turn the tubes over twice. Respect the specified temperature conditions.

## 6. SPECIMEN COLLECTION AND PREPARATION

The assay is performed directly on serum or plasma without heparin. If the assay is to be performed within 24 hours of sample collection, the samples may be stored at 2-8°C. Otherwise, it is better to divide them into aliquots and store deep-frozen (-20°C).

### Dilutions

Should elevated gastrin levels be suspected, the 0 standard found in the kit is used for dilution.

It is recommended that disposable plastic tubes be used when carrying out dilution.

## 7. ASSAY PROCEDURE

### 7.1. Equipment required

Precision micropipettes or similar with disposable tips, permitting the dispensing of 100 µl, 300 µl and 1000 µl (± 1%). Their calibration must be checked regularly.

Distilled water. Disposable plastic tubes. Vortex-type mixer. Multitube centrifuge (1500 g minimum), refrigerated if possible. Tube carrier-trays (preferably able to be turned over).

Gamma scintillation counter calibrated for 125 iodine measurement.

### 7.2. Protocol

The reconstitution of the reagents and their dispensing into the tubes is carried out at room temperature (18-25°C).

The assay requires the following groups of tubes:

T group for the determination of total activity,

0 group for the 0 point of the curve and the determination of binding capacity,

Standard groups to establish the standard curve,

Sx groups for the samples to be assayed.

It is recommended that the assays be performed in triplicate for the T, 0 and standard groups and in duplicate for samples.

Respect the order in which reagents are to be added:

Dispense 100 µl of standards or samples into the corresponding groups of tubes.

Add 100 µl of radiolabeled gastrin to all the tubes.

Add 300 µl of antiserum to the standard and sample tubes.

Gently mix each tube using a Vortex-type mixer.

Incubate for 2 hours at 18-25°C.

Manually shake the content of the immunoprecipitating reagent vial to ensure its homogeneous suspension.

At room temperature, dispense 1 ml of this reagent into all the tubes (except those of the T group).

This step must be carried out as rapidly as possible.

Mix and allow to incubate for 15 minutes at 18-25°C.

Centrifuge all tubes (except those of the T group) at 1500 to 2000 g for 15 minutes, if possible at 2-8°C.

Remove the supernatant either by aspiration or by decanting. The recommended method is to invert the tubes over an "active" sink or a container suitable for holding radioactive solutions. Shake the tubes and leave them inverted for 10 minutes on an absorbent support.

Measure the radioactivity of each tube with a gamma scintillation counter.

## 8. QUALITY CONTROL

Good laboratory practices require that control samples be used in each series of assays to check the quality of the results obtained. These samples must be treated in exactly the same way as the samples to be assayed, and it is recommended that the results be analyzed with appropriate statistical methods.



## 9. RESULTS

For each group of tubes, compute the mean counts after subtracting the background.  
Evaluate the system's liaison capacity.

$$(Bo/T) \% = \frac{\text{Std "0"}}{T} \times 100$$

Calculate the binding percentages of the standards and the samples compared to the 0 standard.

$$(B/Bo) \% = \frac{\text{Std ou Sx}}{\text{Std "0"}} \times 100$$

Draw the standard curve plotting the B/Bo% of the standards as a function of their concentration.

It is recommended to use semi-log coordinates.

Other methods may be used for drawing the curve, in particular by plotting the cpm versus concentration.

Read the samples' values directly from the curve, correcting the read value for the dilution factor if necessary.

**Typical standard curve** (example only): this data must under no circumstances be substituted for results obtained in the laboratory.

Tube groups	Mean (cpm)	B/Bo x 100	Concentration (µU/ml)
T	14371	-	-
0 Standard	6481	100	0
Standard 1	5671	87.5	20
Standard 2	4938	76.2	60
Standard 3	3629	56.0	150
Standard 4	1711	26.4	500
Standard 5	745	11.5	1500
Sample	2074	32.0	440

## 10. PROCEDURAL LIMITATIONS

Samples which show turbidity, haemolysis, hyperlipemia or contain fibrin may give misleading results.

Do not extrapolate sample values beyond the last standard. Dilute the samples concerned and re-assay.

## 11. EXPECTED VALUES

Each laboratory must establish its own range of normal values. Values shown below are given as an example only.

Number of cases	Extreme values (µU/ml)	Mean (µU/ml)
75	28 - 185	68.5

97% of measured values are between 28 and 115 µU/ml.

## 12. SPECIFIC CHARACTERISTICS OF THE ASSAY

### 12.1. Imprecision

This was evaluated using 2 samples with different concentrations assayed either 30 times in the same series or in duplicate in 10 different series.

Sample	Mean (µU/ml)	Within-run (CV %)	Between-run (CV %)
1	45.8	8.9	9.9
2	480	8.4	5.9

### 12.2. Recovery test

Known quantities of gastrin were added to human serum. The recovery percentages for gastrin in the samples ranged between 95 and 105%.

### 11.3. Detection limit

The detection limit is defined as being the smallest detectable concentration different from 0 with a probability of 95%. It has been assessed as being 10 µU/ml.

## ASSAY FLOW CHART

Tubes	Standards, Samples µl	<sup>125</sup> I Gastrin µl	Antiserum µl	Mix. Incubate 2 hours at 18-25°C.	Immuno-precipitating reagent (ml)	Mix. Incubate 15 minutes at 18-25°C. Centrifuge for 15 minutes. Discard the supernatant	Count
T	-	100	-		-		
0	100	100	300		1		
Standards	100	100	300		1		
Sx	100	100	300		1		