Instructions for Use

Pancreatic Polypeptide RIA

IVD

CE

REF **RIA-3027**

Σ **100**

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(230123)

Please use only the valid version of the Instructions for Use provided with the kit.
Verwenden Sie nur die jeweils gültige, im Testkit enthaltene, Gebrauchsanweisung.
Si prega di usare la versione valida delle istruzioni per l'uso a disposizione con il kit.
Por favor, use sólo la versión válida de las instrucciones de uso que se suministran con el kit.

Table of Contents

1 INTENDED USE

Radioimmunoassay for the in vitro quantitative measurement of pancreatic polypeptide (PP) in human serum.

2 CLINICAL BACKGROUND

2.1 Biological activities

Pancreatic polypeptide (PP) is synthesized as an amino-terminal moiety of a precursor peptide. PP isolated from pancreas has 36 amino acid residues with an amidated C-terminal tyrosine. PP is secreted by F-cells of the islets of Langerhans. PP is localized almost entirely in the pancreas although detectable levels throughout gastrointestinal tract have been reported. PP in human plasma is reported to exist in at least four different forms:

PP 1-36, PP 3-36 and two unidentified forms.

PP is released into plasma during stimulation of meal. The physiological role of PP includes inhibition of stimulated gastric and pancreatic exocrine secretions and augmentation of insulin inhibited hepatic glucose production. These actions of PP are mediated by specific receptors. Receptor binding studies have shown that the intact C-terminal tyrosine amide is necessary for biological activity.

2.2 Clinical application

The secretion of PP is stimulated by meal especially protein and fat. PP is also produced by endocrine active tumours in the pancreas and the gastrointestinal tract. These tumours often produce several peptide hormones in the combinations PP-VIP, PP-glucagon or PP-gastrin. Tumours with only PP-secretion have been reported. These tumours may occur at the WDHA or Verner-Morrison syndrome.

Elevated fasting levels of PP in serum are found at the occurrence of PP-producing tumours and endocrine tumours in the pancreas and in the gastrointestinal tract.

3 PRINCIPLE OF THE METHOD

The intended use of these reagents is for assay of PP in human serum.

PP in serum is assayed without extraction by a competitive radioimmunoassay using a rabbit antiserum raised against bovine PP.

PP in calibrators and samples compete with ^{125}I-labelled human PP in binding to the antibodies. ^{125}I-PP binds in a reverse proportion to the concentration of PP in calibrators and samples. Antibody-bound ^{125}I-PP is separated from the unbound fraction using the double antibody-polyethylene glycol precipitation technique. The radioactivity of the precipitates is measured. Human, synthetic PP is used for standardisation.

For professional use within a laboratory.

4 REAGENTS PROVIDED

	Reagents	100 Tests Kit	Reconstitution
ANTISERUM	Rabbit **antiserum** raised against bovine PP. Contains phosphate buffer with human serum albumin and NaN_3.	1 vial lyophilised	**Add** 52 mL distilled water
Ag ^{125}I	**TRACER:** ^{125}Iodine labelled PP in phosphate buffer with human serum albumin and NaN_3. Contains normal rabbit serum	1 vial lyophilised 28 kBq	**Add** 12.5 mL distilled water
Ab PEG	**Double Antibody-PEG**: Goat anti-rabbit Ig antiserum in phosphate buffer with human serum albumin and sodium azide. (<0.1%). Contains polyethylene glycol	1 vial 50 mL	**Ready** for use
DIL BUF	**Calibrator diluent**: PP-free human serum lyophilised. Contains aprotinin. For preparation of PP-working calibrators.	1 vial lyophilised	**Add** 10 mL distilled water
ASS BUF	**Assay buffer**: phosphate buffer containing human serum albumin and sodium azide, (<0.1%). To be used instead of antiserum in the non-specific binding test tubes.	1 vial 5 mL	**Ready** for use
CAL	**PP calibrator** in phosphate buffer containing human serum albumin and sodium azide (<0.1%).	1 vial lyophilised	**Reconstitute** with distilled water by the volume stated on vial label
CONTROL N	**Control** - N = 1 or 2 Lyophilised controls with two different levels of PP.	2 vials lyophilised	**Add** 1 mL distilled water

5 SUPPLIES NOT PROVIDED

The following material is required but not provided in the kit:

1. Distilled water
2. 11-13 x 55 mm disposable tubes, polystyrene
3. Pipettes with disposable tips: 100 µL and 500 µL
4. Pipettes: 1 mL, 5 mL and 10 mL
5. Measuring cylinders: 25 mL and 50 mL
6. Vortex mixer
7. Centrifuge, refrigerated giving a minimum of 1700 × *g*
8. Gamma counter

6 REAGENT PREPARATION

A. Anti-PP

Reconstitute with 52 mL distilled water.
Store at 2 °C - 8 °C.

B. ^{125}I-PP

Reconstitute with 12.5 mL distilled water.
Store at -18 °C or lower if reused.

C. Double antibody-PEG

Ready for use. Mix thoroughly before use.
Store at 2 °C - 8 °C.

D. Calibrator diluent

Reconstitute with 10.0 mL distilled water.
Store at -18 °C or lower if reused.

E. PP-calibrator, 2000 pmol/L
Reconstitute with distilled water by the volume stated on vial label.
Store at -18 °C or lower if reused.
For preparation of PP-working calibrators, see radioimmunoassay procedure.

F. Assay buffer
Ready to use.
Store at 2 °C - 8 °C.

G. Controls
Reconstitute with 1 mL distilled water.
Store at -18 °C or lower if reused.

7 STORAGE AND EXPIRATION DATING OF REAGENTS

Store all reagents at 2 °C - 8 °C before reconstitution and use.

The water used for reconstitution of lyophilised reagents should be distilled in an all-glass apparatus or be of corresponding purity. Dissolve the contents in a vial by gentle inversion and avoid foaming.

The stability of the reagents is found on the labels of the vials.

For lyophilised reagents the expiry dates are valid for the unreconstituted reagents.

Reconstituted reagents are stable for 10 weeks (no longer than to the expiry date) stored correctly.

8 SPECIMEN COLLECTION

Patients should be fasting 10 hours prior to sample collection.

Vein blood is collected in tubes without additives. The sample is allowed to clot.

The serum is separated by centrifugation at +4 °C.

The serum should be frozen within 4 hours and stored at -18 °C or lower until assayed.

Repeated thawing and freezing should be avoided.

9 PROCEDURE

9.1 Handling notes

Reconstitute the reagents as specified. Reagents should be brought to room temperature prior to use. Accuracy in all pipetting steps is essential. All tests (calibrators, controls and samples) should be performed in duplicate.

A complete assay includes:

Calibrators: 7 concentrations; 0, 6.25, 12.5, 25, 50, 100 and 200 pmol/L.

Controls

Samples

Tubes for determination of the **non-specific binding (NSB-tubes)**.

Tubes for determination of the **total radioactivity added (TOT-tubes)**.

9.2 Procedure

1. Reconstitute the reagents according to the instructions.
2. Prepare the PP-working calibrators by dilution of the PP-calibrator 2000 pmol/L with the calibrator diluent according to the following:

   a/ CAL 6: 0.200 mL calibrator 2000 pmol/L + 1.80 mL diluent = 200 pmol/L
   b/ CAL 5: 1.00 mL calibrator 200 pmol/L + 1.00 mL diluent = 100 pmol/L
   c) CAL 4: 1.00 mL calibrator 100 pmol/L + 1.00 mL diluent = 50 pmol/L
   d/ CAL 3: 1.00 mL calibrator 50 pmol/L + 1.00 mL diluent = 25 pmol/L
   e/ CAL 2: 1.00 mL calibrator 25 pmol/L + 1.00 mL diluent = 12.5 pmol/L
   f/ CAL 1: 1.00 mL calibrator 12.5 pmol/L + 1.00 mL diluent = 6.25 pmol/L
   g/ CAL 0: Calibrator diluent = 0 pmol/L.
   Store the calibrator solutions at -18 °C or lower if reused.
3. Pipette 100 µL of the calibrators (0 - 200 pmol/L), samples and controls in their respective tubes.
   Pipette 100 µL of the zero-calibrator in the NSB-tubes.
4. Pipette 500 µL antiserum to all tubes except the NSB- and TOT-tubes.
5. Add 500 µL assay buffer to the NSB-tubes.

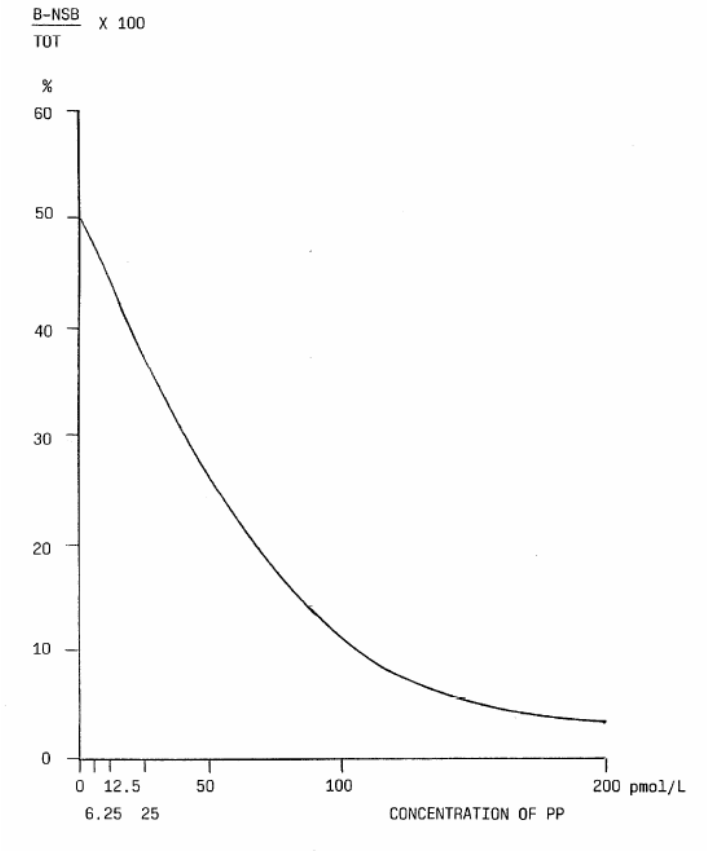
6. Vortex-mix and **incubate for 20 - 24 hours at 2 °C - 8 °C**.
7. Pipette 100 µL ^{125}I-PP to all tubes. The TOT-tubes are sealed and kept aside.
8. Vortex-mix and **incubate for 20 - 24 hours at 2 °C - 8 °C**.
9. Pipette 500 µL double antibody-PEG to all tubes except the TOT-tubes.
   Mix this reagent before pipetting.
10. Vortex-mix carefully and **incubate for 30 - 60 minutes at 2 °C - 8 °C**.
11. Centrifuge the tubes for 15 minutes at +4 °C (minimum 1700 × *g*).
12. Decant the supernatants immediately after centrifugation.
13. Count the radioactivity of the precipitates in a gamma counter (counting time: 2-4 minutes).

10 CALCULATION OF RESULTS

1. Subtract the average count rate (CPM) of the non-specific binding tubes from the count rates (CPM) of the replicates of calibrators, controls and samples.
2. A calibration curve is generated by plotting the precipitated CPM, bound fraction in CPM or % B/TOT against the concentrations of the PP-calibrators.
3. Interpolate the PP concentrations of the samples and controls from the generated calibration curve.
4. The calibration curve and the calculations of the concentrations in samples and controls can also be done by a computer method.

The following data are for illustration only and should never be used instead of the real time calibration curve.

Example of PP calibration curve



11 PERFORMANCE AND LIMITATIONS

11.1 Sensitivity

The lowest detectable concentration is 5 pmol/L. The figure corresponds to a decrease in binding of two x SD of the bound radioactivity in the zero-concentration calibrator.

11.2 Precision

Intra assay variation

Level	Coefficient of variation (% CV)	N
12.9 pmol/L	3.2	16
62.2 pmol/L	4.3	16

Inter assay variation (total variation)

Level	Coefficient of variation (%CV)	N
12.5 pmol/L	16.4	10
66.3 pmol/L	4.2	10

11.3 Accuracy

A mean recovery of 98% was achieved when known amounts of hPP were added to human serum.

	Quantity added	Read value	Theoretical value	% Recovery
Sample 1		39.91		
Sample 1 + (10 pmol/L)	10	55.49	49.9	111%
Sample 1 + (20 pmol/L)	20	62.36	59.9	104%
Sample 1 + (50 pmol/L)	50	95.02	89.9	106%
Sample 1 + (100 pmol/L)	100	129.65	139.9	93%
Sample 2		37.76		
Sample 2 + (10 pmol/L)	10	42.91	47.8	90%
Sample 2 + (20 pmol/L)	20	44.09	57.8	76%
Sample 2 + (50 pmol/L)	50	91.28	87.8	104%
Sample 2 + (100 pmol/L)	100	137.65	137.8	100%

11.4 Specificity

The following cross-reactions have been found

Peptide	Cross-reaction
Pancreatic polypeptide, human	100.0 %
Pancreatic polypeptide, bovine	120 %
Gastric inhibitory peptide, porcine	0.02 %
Cholecystokinin 39, porcine	0.02 %
Secretin, porcine	0.02 %
Gastrin 34, human	< 0.01 %
Gastrin 17, human	< 0.01 %
Glucagon, human / porcine	0.03 %
Insulin, porcine	< 0.01 %
ACTH 1-39, porcine	< 0.003 %
Neuropeptide Y, human	< 0.8 %
Peptide YY, human	< 1.0 %

11.5 Interference

Samples displaying cloudiness, hemolysis, hyperlipemia or containing fibrin may give inaccurate results.

11.6 Dilution

Sera with high analyte concentrations were tested at different dilutions.

Dilution	Expected (pmol/L)	Measured (pmol/L)	% Recovery
Serum 1	41.3		
1/2	20.6	19.5	94%
1/4	10.3	9.9	96%
Serum 2	144.67		
1/2	72.3	71.4	99%
1/4	36.2	37.6	104%
1/8	18.1	19.7	109%

Samples were diluted with diluent buffer.

12 QUALITY CONTROL

In order to completely monitor the consistent performance of the radioimmunoassay there are some important factors which must be checked.

1. **Controls**
   The found concentrations of the controls should be within the limits given on the labels of the vials
2. **Total counts**
   Counts obtained should approximate the expected CPM when adjusted for counter efficiency and radioactive decay. The content of ^{125}I-PP in this kit will give 10500 CPM (-5%, +20%) at activity reference date (counter efficiency = 80 %).
3. **Maximum binding (B_0/TOT)**
   Calculate for each assay the % bound radioactivity in the zero calibrator (B_0/TOT × 100).
4. **Non-specific binding (NSB/TOT)**
   Calculate for each assay the % non-specific binding (NSB/TOT × 100)
   The % non-specific binding should be less than 7 %.
5. **Slope of standard curve**
   For example monitor the 80, 50, and 20% points of the calibration curve for run to run reproducibility.

13 PRECAUTIONS AND WARNINGS

Safety

For in vitro diagnostic use only.

As the regulations may vary from one country to another, it is essential that the persons responsible for the laboratory are familiar with current local regulations, concerning all aspects of radioactive materials of the type and quantity used in this test.

This kit contains components of human origin. They have been tested by immunoassay for hepatitis B surface antigen, antibodies to HCV and for antibodies to HIV-1 and HIV-2 and found to be negative. Nevertheless, all recommended precautions for the handling of blood derivatives, should be observed.

This kit contains ^{125}I (half-life: 60 days), emitting ionizing X (28 keV) and γ (35.5 keV) radiations. Steps should be taken to ensure the proper handling of the radioactive material, according to local and/or national regulations. Only authorized personnel should have access to the reagents.

The following precautions should be observed when handling radioactive materials:

- Radioactive material should be stored in specially designated areas, not normally accessible to unauthorized personnel.
- Handling of radioactive material should be conducted in authorized areas only.
- Care should be exercised to prevent ingestion and contact with the skin and clothing. Do not pipette radioactive solutions by mouth.
- Drinking, eating or smoking should be prohibited where radioactive material is being used.
- Hands should be protected by gloves and washed after using radioactive materials.
- Work should be carried out on a surface covered by disposable absorbing material.
- Spills of radioactive material should be removed immediately, and all contaminated materials disposed as radioactive waste. Contaminated surfaces should be cleaned with a detergent.

The reagents in this kit contain sodium azide. Contact with copper or lead drain pipes may result in the cumulative formation of highly explosive azide deposits. On disposal of the reagents in the sewerage, always flush with copious amounts of water, which prevents metallic azide formation. Plumbing suspected of being contaminated with these explosive deposits should be rinsed thoroughly with 10% sodium hydroxide solution.

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   Gut 17:940-944, 1976.
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   Am J Surgery 151:130-140, 1986.
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    Reversal of abnormal glucose production after pancreatic resection by pancreatic polypeptide administration in man.
    Surgery 104:119-129, 1988.

15 SUMMARY OF THE PROTOCOL

	Total count	**NSB**	**Calibrators (0-6)**	**Controls**	**Samples**
Calibrator 0	-	100 µL	-	-	-
Calibrators	-	-	100 µL	-	-
Controls	-	-	-	100 µL	-
Samples	-	-	-	-	100 µL
Antiserum	-	-	500 µL		
Assay buffer	-	500 µL	-	-	-
Vortex-mix and incubate for 20 - 24 hours at 2 °C – 8 °C					
^{125}I Tracer	100 µL				
Vortex-mix and incubate for 20 - 24 hours at 2 °C – 8 °C					
Double antibody PEG	-	500 µL			
Vortex-mix and incubate for 30 - 60 min at 2 °C – 8 °C					
Centrifuge 15 min (1700 *g* at 4 °C)					
Decant and count the radioactivity of the precipitates					

SYMBOLS USED

Symbol	English	Deutsch	Italiano	Español	Français	Português
CE	European Conformity	CE-Konformitäts-kennzeichnung	Conformità europea	Conformidad europea	Conformité normes européennes	Conformidade Europeia
	Consult instructions for use *	Gebrauchsanweisung beachten *	Consultare le istruzioni per l'uso	Consulte las instrucciones de uso	Consulter les instructions d'utilisation	Consultar as instruções de uso
IVD	*In vitro* diagnostic medical device *	*In-vitro*-Diagnostikum *	Diagnostica in vitro	Diagnóstico in vitro	Diagnostic in vitro	Dispositivo médico para diagnóstico in vitro
REF	Catalogue number *	Katalognummer *	No. di Cat.	No de catálogo	Référence	Número de catálogo
LOT	Batch code *	Chargen-bezeichnung *	Lotto no	Número de lote	No. de lot	Código do lote
Σ	Contains sufficient for <n> tests *	Ausreichend für <n> Prüfungen *	Contenuto sufficiente per "n" saggi	Contenido suficiente para <n> ensayos	Contenu suffisant pour "n" tests	Suficiente para <n> determinações
	Temperature limit *	Temperaturgrenzwerte *	Temperatura di conservazione	Temperatura de conservacion	Temperature de conservation	Limites de temperatura
	Use-by date *	Verwendbar bis *	Data di scadenza	Fecha de caducidad	Date limite d'utilisation	Prazo de validade
	Manufacturer *	Hersteller *	Fabbricante	Fabricante	Fabricant	Fabricante
	Distributor *	Vertriebspartner *	Distributore	Distribuidor	Distributeur	Distribuidor
	Date of manufacture *	Herstellungsdatum *	Data di produzione	Fecha de fabricación	Date de production	Data de fabricação
	Biological risks *	Biologische Risiken *	Rischi biologici	Riesgos biológicos	Risques biologiques	Riscos biológicos
	Caution *	Achtung *	Attenzione	Precaución	Attention	Cuidado
UDI	Unique device Identifier *	eindeutige Produktidentifizierung *	Identificativo unico del dispositivo*	Identificación exclusiva del dispositivo *	Identifiant de dispositif unique*	Identificador único do dispositivo *
RUO	For research use only	Nur für Forschungszwecke	Solo a scopo di ricerca	Sólo para uso en investigación	Seulement dans le cadre de recherches	
Content	Content	Inhalt	Contenuto	Contenido	Conditionnement	Conteúdo
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantità	Volumen/Número	Volume/Quantité	Volume / Quantidade