Instruction

EURIA-Somatostatin

Somatostatin radioimmunoassay

Document No. E-23-0012-09 RUO

July, 2013

For Research Use Only. Not for use in diagnostic procedures.

REF **RB 306 RUO**

Σ 100

PRINCIPLE OF THE METHOD

The intended use is for determination of somatostatin in human plasma. Somatostatin in plasma is extracted with Sep-pak C18 cartridges. The extracts are analysed by a competitive radioimmunoassay using an antiserum to synthetic cyclic somatostatin 14. Somatostatin in standards and samples compete with ^{125}I-labelled somatostatin in binding to the antibodies. ^{125}I-Tyr^{1}-somatostatin binds in a reverse proportion to the concentration of somatostatin in standards and samples. Antibody-bound ^{125}I-Tyr^{1}-somatostatin is separated from the unbound fraction using the double antibody solid phase precipitation technique. The radioactivity of the precipitates is measured.
The result shall not be used for clinical diagnosis or patient management.

PRECAUTIONS

For research use only. Not for use in diagnostic procedures.

As the regulations may vary from one country to another, it is essential that the person responsible for the laboratory is 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.

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 drainpipes 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.

COMPOSITION OF THE REAGENT KIT

The reagents provided in each kit are sufficient for 100 tubes.

1. Anti-somatostatin (Reagent A)

Rabbit antiserum to synthetic cyclic somatostatin. The immunogen was cyclic somatostatin conjugated to bovine thyroglobuline. Lyophilized in 2.0 mL 0.5 M phosphate buffer, pH 7.4, 2.5% human serum albumin, 2.5% EDTA disodium salt and 0.5% sodium azide. Reconstitution in 22 mL distilled water. The reconstituted reagent contains 500 KIU Trasylol® per mL. For 100 tubes. Colour: Yellow.

2. ^{125}I-Somatostatin (Reagent B)

Total radioactivity: 28 KBq or 0.75 μCi . Produced by iodination of Tyr^{1}-somatostatin. HPLC-purified, monoiodinated. Specific activity: 62-77 Mbq/nmol (1700-2100 μCi/nmol). Lyophilized in 2.5 mL 0.5 M phosphate buffer, pH 7.4, 2.5% human serum albumin, 2.5% EDTA disodium salt and 0.5% sodium azide. Reconstitution in 25 mL distilled water. The reconstituted reagent contains 500 KIU Trasylol® per mL. Colour: Blue.

3. Double antibody solid phase (Reagent C)

Anti-rabbit-IgG coupled to cellulose particles in 0.01 M phosphate buffer pH 6.8 with 0.25% Human serum albumin, 0.045% NaCl, 0.05% NaN_3, 0.185% EDTA and 0.05% Tween 80. 11 mL suspension.

4. Assay diluent (Reagent D)

50 mL 0.05 M phosphate buffer, pH 7.4 with 0.25% human serum albumin, 0.25% EDTA disodium salt, 0.05% sodium azide, 0.1% Tween 80 and 500 KIU Trasylol® per mL. To be used for the dilution of the somatostatin standard, reconstitution of sample extracts and instead of antiserum in non-specific binding controls.

5. Somatostatin standard (Reagent E)

5.0 mL, 250 pmol/L, synthetic cyclic somatostatin (MW = 1638.1). Lyophilized in 0.05 M phosphate, pH 7.4 with 0.25% human serum albumin, 0.25% EDTA disodium salt, 0.05% sodium azide and 500 KIU Trasylol® per mL. Reconstitution in 5.00 mL distilled water.

6. Controls (Reagent F-G)

Lyophilized controls with two different levels of somatostatin. 1.00 mL of each control after reconstitution. The somatostatin concentrations are given on the labels of the vials. The controls should be assayed directly without extraction. Contains 0.05% sodium azide.

EQUIPMENT AND REAGENTS REQUIRED BUT NOT PROVIDED

Distilled water.

Methanol, pro analysi.

Hydrochloric acid, 1 M.

Acetic acid, pro analysi.

11-13 x 55 mm disposable test tubes (polystyrene).

Pipettes with disposable tips: 100, 200, 400 and 1000 µL.

Glass pipettes: 1.00, 5.00 mL.

Vortex mixer.

Speedvac evaporator or freeze drier (for evaporation of methanol).

Centrifuge, refrigerated, giving a minimum of 1700 x g.

Gamma counter.

Sep-pak C18 cartridges.

REAGENT PREPARATION AND STORAGE

Store all reagents at 2-8° C before reconstitution and use. The water used for reconstitution of the lyophilized 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 label of the vials. For lyophilized reagents the expiry date is valid for the unreconstituted reagents. Reconstituted reagents are stable for 10 weeks or until the expiry date is reached when stored according to the instructions.

Reagent A: Anti-somatostatin
Reconstitute with 22 mL distilled water.
Store at 2-8° C.

Reagent B: ^{125}I-Somatostatin
Reconstitute with 25 mL distilled water.
Store at -18° C or lower if reused.

Reagent C: Double antibody solid phase
Ready for use. Mix continuously during pipetting of this reagent.
Store at 2-8° C.

Reagent D: Assay diluent
Ready for use.
Store at 2-8° C.

Reagent E: Somatostatin standard, 250 pmol/L
Reconstitute with 5.00 mL distilled water.
Store at -18° C or lower if reused.

Reagent F-G: Controls
Reconstitute each vial with 1.00 mL distilled water.
Store at -18° C or lower if reused.

SPECIMEN COLLECTION

Blood is collected in tubes containing EDTA and Trasylol® (2500 KIU Trasylol for 5 mL blood). The sample is cooled in an ice-bath immediately. Plasma is separated by centrifugation at +4° C. The plasma should be frozen within 30 minutes and stored at -20° C or lower until assayed. Store no longer than 3 to 4 weeks. For longer time store at -70° C. Repeated freezing and thawing must be avoided!!!

ASSAY PROCEDURE

The assay is performed in two steps:

I. Extraction of plasma samples
II. Radioimmunoassay of extracts

For an overview of the RIA procedure see page 12.

A complete assay includes:

Standard (St-tubes): 7 concentrations: 0, 3.9, 7.8, 15.6, 31.3, 62.5 and 125 pmol/L.

Controls (C-tubes): For recovery control (C_{REC}) and radioimmunoassay control (C_{RIA}). The recovery of somatostatin in the extraction procedure is determinated by analysing a sample with exactly known concentration of somatostatin.

Samples (S-tubes

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

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

PERFORMANCE

I. Extraction of plasma samples

The described extraction procedure is based on the use of Sep-pak® C18 cartridges available from Millipore. The procedure has been tested with Sep-pak C18 cartridge, product no.
WAT 020515.
It is important that the recovery is controlled under the user's own experimental conditions.

1. Thaw the samples immediately before starting the extraction. Store at 2-8° C until adding 1 M HCl.

2. Add 100 µl 1M HCl per mL of sample e.g. 500 µl 1M HCl to 5.0 mL sample. Vortex mix carefully.

3. The Sep-pak cartridge is wetted with 5 mL methanol.

4. Wash the Sep-pak cartridge with 20 mL distilled water.

5. Apply 1.00 mL plasma sample (to which has been added 0.1 mL 1M HCl per mL) on the Sep-pak cartridge. The flowrate should not exceed 1 mL/10 seconds.

6. Wash with 20 mL 4% acetic acid in distilled water.

7. Elute the somatostatin with 2.0 mL methanol. The flowrate should not exceed 1 mL/10 seconds.
   Collect the eluate in a 10 mL glass tube.

8. Evaporate to dryness in a Speed vac evaporator.

9. Dissolve the extracted somatostatin in 1.00 mL assay diluent (Reagent D). Vortex mix and allow the sample to stay for 30 minutes before assay with the radioimmunoassay procedure.

RECOVERY CONTROLS

For the determination of the recovery in the extraction procedure prepare controls as follows:

To 800 µL blood donor EDTA-plasma, to which previously has been added 0.1 mL 1M HCl per mL plasma, add exactly 200 µl of the somatostatin standard 250 pmol/L (Reagent E). The concentration will be 50 pmol/L (control a). Extract the control according to the procedure described for samples.
To another 800 µl volume of the same blood donor plasma (with 0.1 mL 1M HCl per mL plasma) add 200 µl of assay diluent (Reagent D). Extract the control according to the procedure described for samples (control b). Control b is used for correction for endogenous somatostatin in the calculation of the recovery of added somatostatin.

II. Radioimmunoassay of extracts

1. Reconstitute the reagents according to the instructions.

2. Prepare the somatostatin working standards by dilution of the 250 pmol/L standard (Reagent E) with the assay diluent (Reagent D) according to the following:

   a/ 1.00 mL standard 250 pmol/L + 1.00 mL assay diluent = 125 pmol/L
   b/ 1.00 mL standard 125 pmol/L + 1.00 mL assay diluent = 62.5 pmol/L
   c/ 1.00 mL standard 62.5 pmol/L + 1.00 mL assay diluent = 31.3 pmol/L
   d/ 1.00 mL standard 31.3 pmol/L + 1.00 mL assay diluent = 15.6 pmol/L
   e/ 1.00 mL standard 15.6 pmol/L + 1.00 mL assay diluent = 7.8 pmol/L
   f/ 1.00 mL standard 7.8 pmol/L + 1.00 mL assay diluent = 3.9 pmol/L
   g/ Assay diluent = 0 pmol/L.

   Store the standard solutions a-g and reagent E at -18° C or lower if reused.

3. Pipette 100 µL of standards a-g, controls and reconstituted sample extracts and reconstituted control extracts in their respective tubes (duplicates). Pipette 100 µl of the zero-standard (assay diluent) in the NSB-tubes (duplicates).

4. Add 200 µL anti-somatostatin (Reagent A) to all tubes except the NSB- and TOT-tubes.

5. Add 200 µL assay diluent (Reagent D) to the NSB-tubes.

6. Vortex mix and incubate for 20-24 hours at 2-8° C.

7. Add 200 µL ^{125}I-somatostatin (Reagent B) to all tubes. The TOT-tubes are sealed and kept aside.

8. Vortex mix and incubate for 20-24 hours at 2-8° C.

9. Add 100 µL double antibody, solid phase (Reagent C) to all tubes except the TOT-tubes (stir continuously during pipetting).

10. Vortex mix and incubate for 30-60 minutes at 2-8° C.

11. Centrifuge the tubes for 15 minutes at +4° C (1700 x g).

12. Decant the supernatant immediately after centrifugation.

13. Count the radioactivity of the pellets in a gamma counter (counting time 2-4 min).

CALCULATION OF RESULTS

1. Subtract the average count rate (CPM) of the non-specific binding from the count rate (CPM) of the replicates of standards, controls and samples.

2. A standard curve is generated by plotting the precipitated CPM, bound fraction (in CPM or %B/TOT) against the concentrations of the somatostatin standards.

3. Interpolate the somatostatin concentrations in the samples and controls from the generated standard curve.

4. Calculate the recovery of somatostatin in the recovery controls.
   % recovered somatostatin =

   $$\frac{\text{(Mean conc. of control a - Mean conc. of control b) x 100}}{\text{50 (= added concentration)}}$$

5. Correct the sample concentrations for the % recovery.
   Correct the sample concentrations for the increase of volume when adding 1M HCl.
   Multiply with a factor of 1.10.

6. The standard curve and the calculation of the concentrations of the samples can also be done by a computer method. A spline method may be used.

ASSAY CHARACTERISTICS

Sensitivity
The sensitivity calculated from a decrease in binding of 2 SD in the zerostandard is 6 pmol/L.

Recovery
The mean recovery in the extraction procedure is 79 ± 10% (obtained in this laboratory).

Precision
Intra assay variation

Level	Coefficient of variation (CV	N
16.4 pmol/L	8.3%	20
57.3 pmol/L	2.8%	20

Inter assay variation (= total variation)

Level	Coefficient of variation	N
17.3 pmol/L	6.4%	7
57.7 pmol/L	3.3%	7

Specificity
The following cross reactions have been found:

Polypeptide	Cross reaction
Somatostatin, cyclic	100.0 %
Tyr^{1}-somatostatin	100 %
Linear somatostatin	50 %
Tyr^{11}-somatostatin	38 %
Des-ala-gly-somatostatin	25 %

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

SOMATOSTATIN CONCENTRATION IN HUMAN PLASMA

The somatostatin concentration in normal fasting subjects assayed with these reagents was <16 pmol/L.

QUALITY CONTROL

In order for the laboratory 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 (Reagent F and G) should be within the limits given on the labels of the vials.

2. Recovery control
The recovery should be at least 60% for a valid assay. It is important that the recovery is controlled under the user's own experimental conditions. The recovery obtained at the product development laboratory was 79 $\pm$ 10%.

3. Total counts
Counts obtained should approximate the expected CPM when adjusted for counter efficiency and radioactive decay. The content of ^{125}I-Tyr^{1}-somatostatin in this kit will give a total counts in the assay (TOT) of 10500 CPM (+ 20%, -5%) at the activity reference date (counter efficiency = 80%).

4. Maximum binding (Bo/TOT)

Calculate for each assay the % bound radioactivity of the zero standard ($\frac{Bo}{TOT} \times 100$).

$\frac{Bo}{TOT} \times 100$ is generally 40-55% at the activity reference date.

$\frac{Bo}{TOT} \times 100$ may have decreased 2-5% at the expiry date of the kit.

5. Non-specific binding (NSB/TOT)

Calculate for each assay the non-specific binding: $\frac{NSB}{TOT} \times 100$

$\frac{NSB}{TOT} \times 100$ is less than 6% if decanting is made properly.

6. Shape of standard curve

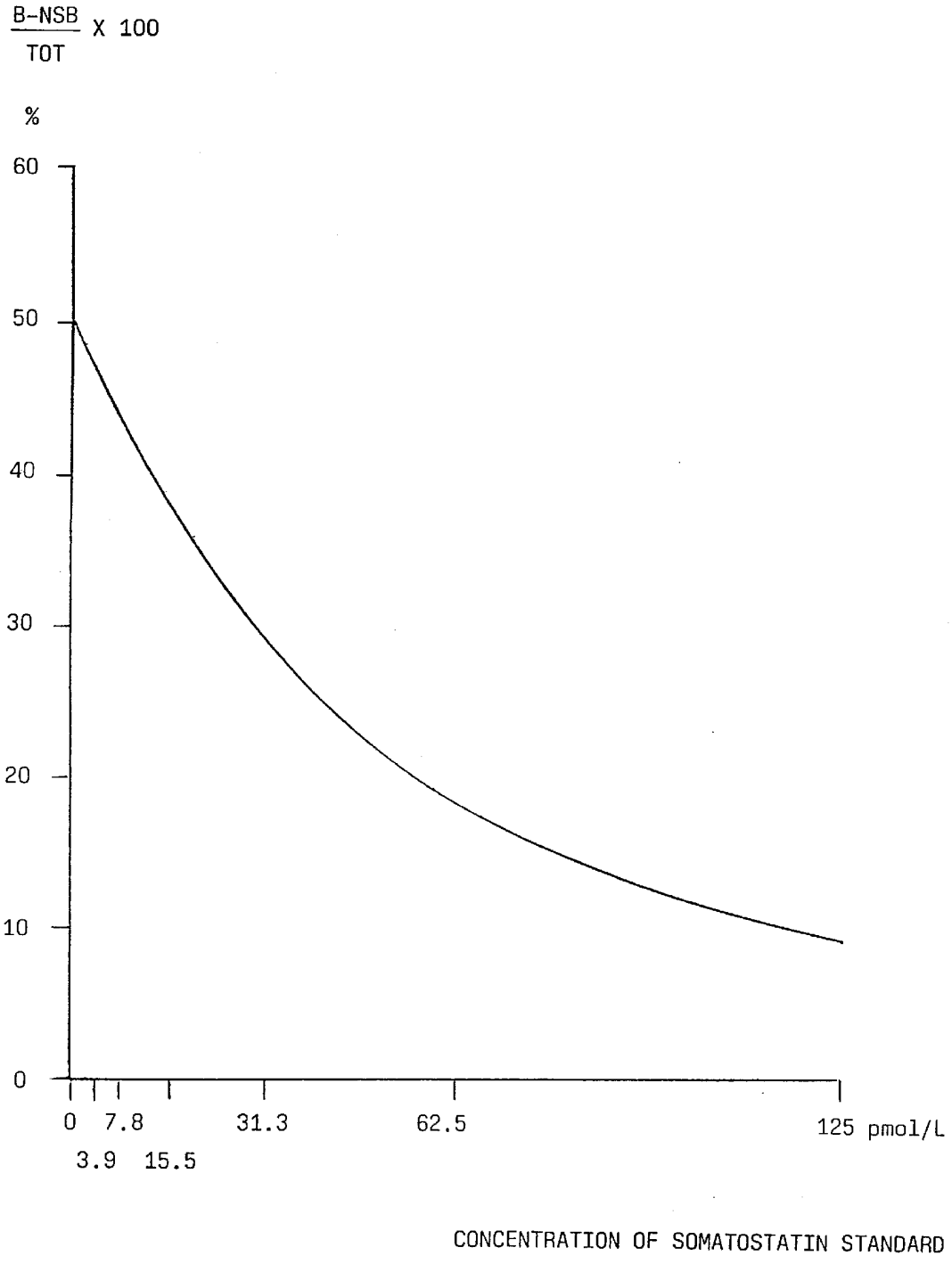
For example, monitor the 80, 50 and 20% points of the standard line for run to run reproducibility.

OUTLINE OF THE RIA PROCEDURE

Type of tubes	Tube no	Standard sample or control	Anti-somato-statin (A)	Assay diluent (D)		^{125}I-somato-statin (B)		Double antibody solid phase (C)	
TOT	1- 2	-	-	-	Vortex-mix and incubate for 20-24 hours at 2-8° C.	200 µL	Vortex-mix and incubate for 20-24 hours at 2-8° C.	-	Vortex-mix and incubate for 30-60 min. at 2-8° C. Centrifuge 15 min. at 1700 x g at +4° C. Decant and count the radioactivi-ty of the pellets.
NSB	3- 4	-	-	300 µL		200 µL		100 µL	
Stand 0	5- 6	100 µL	200 µL	-		200 µL		100 µL	
Stand 3.9	7- 8	100 µL	200 µL	-		200 µL		100 µL	
Stand 7.8	9-10	100 µL	200 µL	-		200 µL		100 µL	
Stand 15.6	11-12	100 µL	200 µL	-		200 µL		100 µL	
Stand 31.3	13-14	100 µL	200 µL	-		200 µL		100 µL	
Stand 62.5	15-16	100 µL	200 µL	-		200 µL		100 µL	
Stand 125	17-18	100 µL	200 µL	-		200 µL		100 µL	
$Control_{RIA}$ (F)	19-20	100 µL	200 µL	-		200 µL		100 µL	
$Control_{RIA}$ (G)	21-22	100 µL	200 µL	-		200 µL		100 µL	
$Control_{REC}$ (a)	23-24	100 µL	200 µL	-		200 µL		100 µL	
$Control_{REC}$ (b)	25-26	100 µL	200 µL	-		200 µL		100 µL	
Sample 1	27-28	100 µL	200 µL	-		200 µL		100 µl	
Sample 2	29-30	100 µL	200 µL	-		200 µL		100 µL	

Table 1.

EXAMPLE OF SOMATOSTATIN STANDARD CURVE



REFERENCES

1. Peeters, Theo L., Depraetere, Y. and Van Trappen, R.
Simple extraction method and radioimmunoassay for somatostatin in human plasma.
Clin Chem 27:888 (1981).

2. Berg, J., Nilsson, K., Ekman, R. and Giovanella, B.
Establishment and characterization of cell lines from human small cell and large cell carcinomas of the lung.
Acta Path Microbiol et Immunologica Scand 93A:133-147, 1985.

3. Widerlöv, E., Walleus, H., Lindström, L.H., Karlsson, I., Nemeroff, C.B., Rehfeld, J.F. and Ekman, R.
Neuropeptide alterations in cerebrospinal fluid and plasma from psychiatric patients.
Proceedings of the IV world congress of biological psychiatry Philadelphia 1985.
Eds.: Shagass et al. Elsevier. New York (1986), pp 856-858.

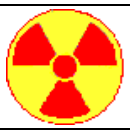
4. Somatostatin
Wass, J.A.H.
In Endocrinology volume no. 1, pp 152-166.
Editor: De Groot, Leslie J.
W.B. Saunders Company, Philadelphia, 1989.

5. Penman, E., Wass, J.A.H., Lund, A. et al.
Development and validation of the specific radioimmunoassay for somatostatin in human plasma.
Ann Clin Biochem 16:15-25, 1979.

6. Saito, H., Ogawa, T., Ischmaru, K., et al.
Plasma somatostatin in normal and diseased states.
Program of the VIth International congress of endocrinology,
Melbourne, 1980. Abst. 75.

7. Arimura, A., Lundqvist, G., Rothman, J. et al.
Radioimmunoassay of somatostatin.
Metabolism 27 (Suppl 1):1139-1144, 1978.

EXPLANATION OF SYMBOLS

LOT	Batch code.
REF	Catalogue number.
	Use by date.
	Temperature limit.
	Date of manufacture.
	Contains radioactive substances.
	Biological risks.
	Consult instructions for use.
	Manufacturer.
 100	Contains sufficient for 100 tests.

REAG A Ab	Anti- somatostatin.
REAG B Ag ^{125}I	^{125}I- Somatostatin.
REAG C DASP	Double antibody solid phase.
REAG D DIL	Assay diluent.
REAG E CAL 250	Somatostatin standard, 250 pmol/L.
REAG F CONTROL	Control.
REAG G CONTROL	Control.

EURO DIAGNOSTICA AB
Lundavägen 151, SE-212 24 Malmö, Sweden
Phone: +46 40 53 76 00, Fax: +46 40 43 22 88
E-mail: info@eurodiagnostica.com
www.eurodiagnostica.com