Somatostatin - RIA

RB306RUO

DIAsource ImmunoAssays S.A. - Rue du Bosquet, 2 - B - 1348 Louvain-la-Neuve – Belgium

History

Summary of change:

Previous Version: 191011-1	Current Version: 200224-1
LOT	Version :
	Addition of the following sentence at the end of the English IFU: "Other translations of this Instruction for Use can be downloaded from our website: https://www.diasource-diagnostics.com/"

en

Read entire protocol before use.

Somatostatin RIA

I. INTENDED USE

Radioimmunoassay for the *in vitro* quantitative measurement of Somatostatin in human plasma.
For Research use only. Not for use in diagnostic procedures.

II. GENERAL INFORMATION

A. Proprietary name : DIAsource Somatostatin RIA

B. Catalog number : RB306RUO : 100 tests

C. Manufactured by : DIAsource ImmunoAssays S.A.
Rue du Bosquet, 2, B-1348 Louvain-la-Neuve, Belgium.

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III. PRINCIPLES OF THE METHOD

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-Tyr1-somatostatin binds in a reverse proportion to the concentration of somatostatin in standards and samples. Antibody-bound ^{125}I-Tyr1-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.

IV. REAGENTS PROVIDED

Reagents	100 Tests Kit	Colour Code	Reconstitution
ANTISERUM Rabbit antiserum to synthetic cyclic somatostatin : the immunogen was cyclic somatostatin conjugated to bovine thyroglobuline. in phosphate buffer with sodium azide, human serum albumin, disodium salt and aprotinin.	1 vial lyophilised	Blue	**Add** 22 ml distilled water
Ag ^{125}I TRACER: ^{125}Iodine labelled somatostatin in phosphate buffer human serum albumin, EDTA disodium salt, sodium azide and aprotinin.	1 vial lyophilised 28 kBq	Red	**Add** 25 ml distilled water
DASP Double antibody-PEG : goat anti-rabbit-IG antiserum in phosphate buffer with human serum albumin, EDTA disodium salt, NaCl, NaN_3 and Tween 80.	1 vial 11 mL	Green	**Ready** for use
ASS BUF Diluent : Phosphate buffer containing human serum albumin, EDTA disodium salt, sodium azide, Tween 80 and aprotinin. To be used for the preparation of somatostatin standards, reconstitution of sample extracts and instead of antiserum in non-specific binding controls.	1 vial 50 mL	Black	**Ready** for use
CAL Somatostatin standard in phosphate buffer containing human serum albumin, EDTA disodium salt, sodium azide (<0.1%) and aprotinin.	1 vial lyophilised	Yellow	**Reconstitute** with distilled water by the volume stated on vial label
CONTROL N Controls - N = 1 or 2 Contains sodium azide.(<0.1%).	2 vials lyophilised	Silver	**Add** 1 mL distilled water

V. SUPPLIES NOT PROVIDED

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

1. Distilled water.
2. Methanol, pro analysis.
3. Hydrochloric acid, 1 M.
4. Acetic acid, pro analysis.
5. 11-13 x 55 mm disposable test tubes (polystyrene).
6. Pipettes with disposable tips: 100, 200, 400 and 1000 µL.
7. Pipettes: 1 mL, 5 mL.
8. Vortex mixer.
9. Speedvac evaporator or freeze drier (for evaporation of methanol).
10. Centrifuge, refrigerated, giving a minimum of 1700 x g.
11. Gamma counter.
12. Sep-pak C18 cartridges.

VI. REAGENT PREPARATION

A. Anti-Somatostatin : Reconstitute with 22 mL of distilled water. Store at 2-8° C.
B. ^{125}I-somatostatin : Reconstitute with 25 mL of distilled water. Store at -18° C or lower if reused.
C. Double antibody solid phase : Ready for use. Mix continuously during pipetting of this reagent. Store at 2-8° C.
D. Assay diluent : Ready for use. Store at 2-8° C.
E. Somatostatin standard : Reconstitute with distilled water by the volume stated on vial label. Store at -18° C or lower if reused.
F. Controls : Reconstitute with 1 mL distilled water. Store at -18° C or lower if reused.

VII. STORAGE AND EXPIRATION DATING OF REAGENTS

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 un-reconstituted reagents. Reconstituted reagents are stable for 10 weeks or until the expiry date is reached when stored according to the instructions.

VIII. SPECIMEN COLLECTION

Blood is collected in 10 mL test tubes containing EDTA and aprotinin 5000 KIU/mL (Trasylol® or equivalent). 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!!!

IX. PROCEDURE

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. 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. The concentration will be 50 pmol/L. 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. Extract the control according to the procedure described for samples. Control 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 with the assay diluent 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 250 pmol/L standard 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 to all tubes except the NSB- and TOT-tubes.
5. Add 200 µL assay diluent to the NSB-tubes.
6. Vortex mix and incubate for 20-24 hours at 2-8° C.
7. Add 200 µL ^{125}I-somatostatin 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 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).

XI. CALCULATION OF RESULTS

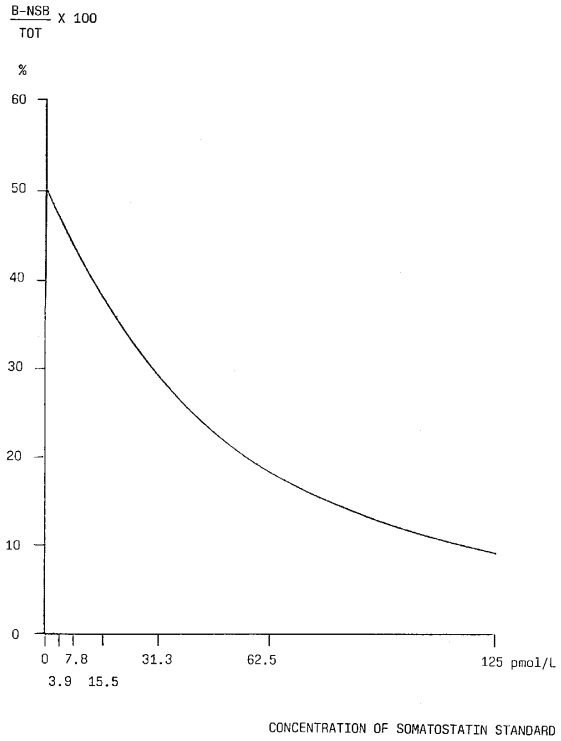
1. Subtract the average count rate (CPM) of the non-specific binding from the count rate (CPM) of the replicates of standards, controls and samples.
2. A standard curve is generated by plotting the precipitated CPM, bound fraction (in CPM or %B/TOT) against the concentrations of the Somatostatin standards.
3. Interpolate the Somatostatin concentrations of the samples and controls from the generated standard curve.
4. Calculate the recovery of somatostatin in the recovery controls.
   % recovered somatostatin =

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

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 in the samples can also be done by a computer method. A spline method may be used.

XII. TYPICAL DATA

EXAMPLE OF SOMATOSTATIN STANDARD CURVE



XIII. PERFORMANCE AND LIMITATIONS

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

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

C. Precision

Intra assay variation:

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

Total variation (Inter assay):

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

D. 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%

D. Interference

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

XIV. SOMATOSTATIN CONCENTRATION IN HUMAN PLASMA

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

XV. INTERNAL 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 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 ± 10%.

3. Total counts

Counts obtained should approximate the expected CPM when adjusted for counter efficiency and radioactive decay. The content of ^{125}I-Tyr1-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 in the zero-standard:

$$\frac{Bo}{TOT} \times 100$$

5. Non-specific binding (NSB/TOT)

Calculate for each assay the % non-specific binding:

$$\frac{NSB}{TOT} \times 100$$

The non-specific binding should be less than 6%.

5. Shape of standard curve

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

XVI. PRECAUTIONS AND WARNINGS

Safety

For research use only.

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.

XVII. BIBLIOGRAPHY

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XVIII. SUMMARY OF THE PROTOCOL

	Total count	NSB	Calibrators (0-6)	Controls	Samples
Calibrator	-	-	**100 µl**	-	-
Controls	-	-	-	**100 µl**	-
Samples	-	-	-	-	**100 µl**
Anti-somatostatin	-	-	**200 µl**		
Assay diluent	-	**300 µl**	-	-	-
Vortex-mix and incubate for 20-24 hours at 2-8°C.					
^{125}I Tracer	**200 µl**				
Vortex-mix and incubate for 20-24 hours at 2-8°C.					
Double antibody solid phase	-	**100 µl**			
Vortex-mix and incubate for 30-60 min at 2-8°C.					
Centrifuge 15 min (1700 g; 4°C)					
Decant and count the radioactivity of the pellets					

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