α-MSH-RIA

RB303RUO

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

Version : 240215

Date of issue : 15/02/2024

Revision date: 15/02/2024

History

Summary of change:

Current Version: 230123	New Version: 240215
-	***XV PRECAUTIONS AND WARNINGS*** This kit contains 125I (half-life: 60 days), emitting ionizing X (28 keV) and ? (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:
V. REAGENTS PROVIDED Contains color code	***V. REAGENTS PROVIDED*** color code removed

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Read entire protocol before use.

α-MSH RIA

I. INTENDED USE

Radioimmunoassay for the *in vitro* quantitative measurement of α-melanocyte stimulating hormone (α-MSH) in human plasma or cerebrospinal fluid.
For Research use only. Not for use in diagnostic procedures.

II. GENERAL INFORMATION

A. Proprietary name : DIAsource α-MSH RIA

B. Catalog number : RB303RUO : 100 tests

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

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III. BACKGROUND

α-Melanocyte stimulating hormone (α-MSH) is a 13 amino acids peptide with a molecular weight of 1665. The N-terminal serin is acetylated and the C-terminal valine is amidated. The amino acid sequence of α-MSH is identical to ACTH 1-13 in man.
α-MSH is derived from pro-opiomelanocortin, a precursor protein which contains, within its structure, the sequences of other melanotropic peptides like α-, ß-MSH and ACTH.
α-MSH stimulates melanosome dispersion within dermal melanocytes and melanin biosynthesis within epidermal melanocytes.
α-MSH is a potent modulator of fever and inflammation. Plasma α-MSH increases in human subjects with high fever caused by endotoxin administration. The average plasma α-MSH level has been found higher in subjects with AIDS than in control subjects.
The result shall not be used for clinical diagnosis or patient management.

IV. PRINCIPLES OF THE METHOD

α-MSH is analysed by the competitive radioimmunoassay using an antiserum to an α-MSH-albumin conjugate. α-MSH in standards and samples compete with ^{125}I-labelled α-MSH in binding to the antibodies.
^{125}I-α-MSH binds in a reverse proportion to the concentration of α-MSH in standards and samples. In order to increase the sensitivity of the assay a sequential assay with delayed addition of ^{125}I-α-MSH is performed. Antibody-bound ^{125}I-α-MSH is separated from the free fraction using the double antibody polyethylene glycol precipitation technique. The radioactivity of the precipitates is measured. The antiserum used in this kit is directed to the C-terminal part of the α-MSH molecule and shows no cross-reactivity with adrenocorticotropic hormone.

V. REAGENTS PROVIDED

Reagents	100 Tests Kit	Reconstitution
[ANTISERUM] Anti-α-MSH : Rabbit antiserum raised against α-MSH, conjugated to bovine serum albumin in phosphate buffer with sodium azide and aprotinin.	1 vial lyophilised	**Add** 22 ml distilled water
Ag | ^{125}I TRACER: ^{125}Iodine labelled α-MSH in phosphate buffer with normal rabbit serum, EDTA disodium salt, sodium azide and aprotinin.	1 vial lyophilised 28 kBq	**Add** 25 ml distilled water
[Ab|PEG] Double antibody-PEG : goat anti-rabbit-IG antiserum in phosphate buffer with human serum albumin, EDTA disodium salt, NaN_3 and polyethylene glycol.	1 vial 50 mL	**Ready** for use
[DIL|BUF] Diluent : Phosphate buffer containing human serum albumin, EDTA disodium salt, sodium azide and aprotinin. To be used for the preparation of α-MSH standards.	1 vial 25 mL	**Ready** for use
[CAL] α-MSH standard in phosphate buffer containing human serum albumin, EDTA disodium salt, sodium azide (<0.1%) and aprotinin.	1 vial lyophilised	**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	**Add** 1 mL distilled water

VI. 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, glass (for dilution of the standard).
3. 11-13 x 55 mm disposable tubes, polystyrene (for the radioimmunoassay procedure).
4. Pipettes: 1 and 5 mL.
5. Measuring cylinder: 25 mL.
6. Pipettes with disposable tips: 100, 200 and 500 µl.
7. Vortex mixer.
8. Centrifuge, refrigerated, giving minimum 1700 x g.
9. Gamma counter.

VII. REAGENT PREPARATION

A. Anti-α-MSH : Reconstitute with 22 mL of distilled water. Store at 2-8° C.
B. ^{125}I-α-MSH : Reconstitute with 25 mL of distilled water. Store at -18° C or lower if reused.
C. Double antibody PEG : Ready for use. Mix thoroughly before use. Store at 2-8° C.
D. Diluent : Ready for use. Store at 2-8° C.
E. α-MSH 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.

VIII. STORAGE AND EXPIRATION DATING OF REAGENTS

Store all reagents at 2-8° C before reconstitution and use. The water used for reconstitution of lyophilized reagents should be destilled 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 dates are valid for the unreconstituted reagents. Reconstituted reagents are stable for 10 weeks, or until the expiry date is reached, stored correctly.

IX. SPECIMEN COLLECTION

Blood is collected in tubes containing EDTA and aprotinin (Trasylol® or equivalent) (5000 KIU aprotinin (Trasylol® or equivalent) in a 10 mL vacutainer). The sample is cooled in an ice-bath immediately. Plasma is separated by centrifugation at 4° C. The plasma should be frozen within 1 hour and stored at -18° C or lower until assayed. Repeated freezing and thawing should be avoided.

X. PROCEDURE

A. Handling notes

Accuracy in all pipetting steps is essential. The assay is performed with duplicates (standards, controls, samples, control tubes for non-specific binding and total activity).
A complete assay includes:
Standard : 7 concentrations: 0, 4.7, 9.4, 18.8, 37.5, 75 and 150 pmol/L.
Samples
Controls : 2 controls with known concentrations of α-MSH for quality control.
Tubes for determination of the non-specific binding for standards and samples.
Tubes for determination of the total radioactivity added.

B. Procedure

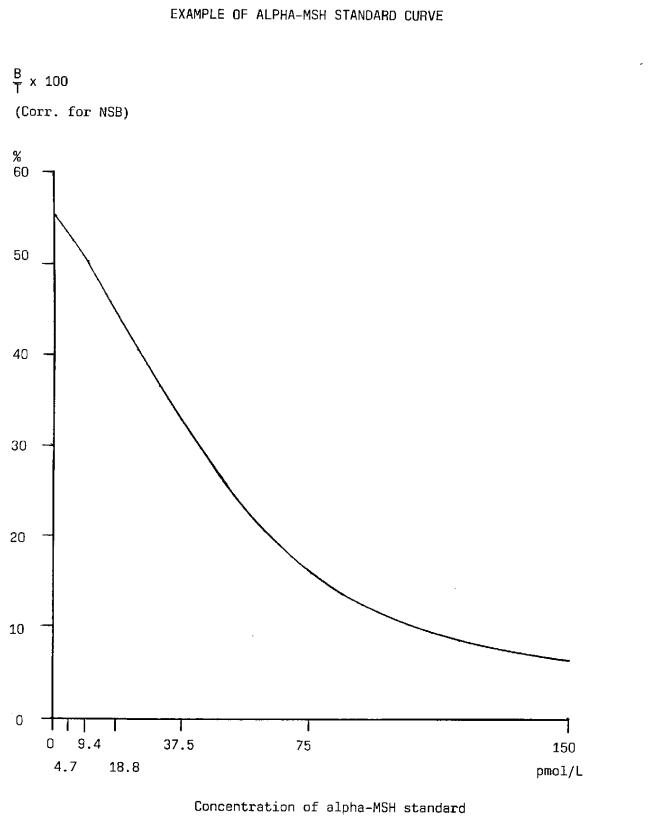
1. Reconstitute the reagents according to the instructions.
2. Prepare the α-MSH working standards by dilution of the α-MSH standard 300 pmol/L with diluent according to the following (use glass-tubes for standard preparation):
   a/ 1.00 mL standard 300 pmol/L + 1.00 mL diluent = 150 pmol/L.
   b/ 1.00 mL standard 150 pmol/L + 1.00 mL diluent = 75 pmol/L.
   c/ 1.00 mL standard 75 pmol/L + 1.00 mL diluent = 37.5 pmol/L.
   d/ 1.00 mL standard 37.5 pmol/L + 1.00 mL diluent = 18.8 pmol/L.
   e/ 1.00 mL standard 18.8 pmol/L+ 1.00 mL diluent = 9.4 pmol/L
   f/ 1.00 mL standard 9.4 pmol/L + 1.00 mL diluent = 4.7 pmol/L
   g/ Diluent = 0 pmol/L.
   Store the standard solutions at -18° C or lower if reused.
3. Pipette 100 µL of standards a-g (0-150 pmol/L), samples and controls in their respective tubes. Pipette 100 µL of the zero-standard in the NSB-tubes.
4. Add 200 µL anti-α-MSH to all tubes except the NSB- and TOT-tubes.
5. Add 200 µL diluent to the NSB-tubes.
6. Vortex-mix and incubate for 20-24 hours at 2-8° C.
7. Add 200 µL ^{125}I-α-MSH 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 500 µL double antibody-PEG to all tubes except the TOT-tubes (mix this reagent before 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 supernatants immediately after centrifugation.
13. Count the radioactivity of the precipitates in a gamma counter (counting time 2-4 minutes).

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 α-MSH standards.
3. Interpolate the α-MSH concentrations of the samples and controls from the generated standard curve.

4. 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



XIII. PERFORMANCE AND LIMITATIONS

A. Sensitivity

The sensitivity calculated from a decrease in binding of 2 SD in the zero standard is 3 pmol/L.

B. Precision

Intra assay variation:

Level	Coefficient of variation (%CV)
16.2 pmol/L	11.8
33.6 pmol/L	4.7
77.7 pmol/L	2.9

Total variation (Inter assay):

Level	Coefficient of variation (%CV)
16.5 pmol/L	13.0
37.8 pmol/L	8.4
79.6 pmol/L	4.0

C. Specificity

The following cross reactions have been found:

Peptide	Cross reaction
α-MSH	100.0%
Des-acetyl-α-MSH	100.0%
Des-amido-α-MSH	<0.002%
ACTH 1-13	<0.002%
ACTH 1-24	<0.002%
ACTH 1-39	<0.002%
Beta-MSH	<0.002%
Gamma-MSH	<0.002%

D. Interference

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

XIV. INTERNAL QUALITY CONTROL

In order to enable the laboratory to completely monitor the consistent performance of the assay, the following important factors should be checked.

1. The found concentrations of the 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-α-MSH in this kit will give 10.500 CPM
(-5, +20%) at the reference date (counting efficiency: 80%).

3. Maximum binding (Bo/TOT)

Calculate for each assay the % bound radioactivity in the zero-standard:

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

4. 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. Slope of standard curve

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

XV. 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 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.

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-α-MSH	-	-	**200 µl**		
Diluent	-	**200 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 PEG	-	**500 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 precipitates					

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