

Product information

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Aldosterone RIA

RUO

REF IB78247

Σ 96

1. PRINCIPLE OF THE ASSAY

The present method is based on a competitive radioimmunoassay (RIA). During the incubation, the sample/calibrator aldosterone competes with the aldosterone labeled with Iodine 125 (tracer) for the specific sites of the antiserum coated on the tubes. Separation is based on the use of antibody coated tubes, where the anti-aldosterone antiserum is fixed on the tube walls. After aspiration, the radioactivity in the tubes is measured in a gamma counter. The degree of binding will be inversely proportional to the sample/calibrator hormone concentration.

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

2. REAGENTS PROVIDED WITH THE KIT

- The reagents are sufficient for 96 determinations.
- Store the kit and reagents at 2-8°C.
- The expiration date of each reagent is shown on the label.

Reagents	96 Tests Kit	Reconstitution
SORB CT Tubes coated with anti-Aldosterone antibody	2 x 48	Ready to Use
TRACER I-125 TRACER : ¹²⁵ I-Aldosterone in phosphate buffer. Preservative: NaN ₃ (<0.1%)	1 Vial 50ml 85 kBq	Ready to Use
CAL 0 LYO Calibrator zero Aldosterone in human serum Preservative: NaN ₃ (<0.1%)	2 Vials Lyophilized	Add 3 mL distilled water
CAL 1 – 6 LYO Calibrator – N = 6 Aldosterone in human serum Preservative: NaN ₃ (<0.1%)	6 Vials Lyophilized	Add 2 mL distilled water
CONTROL LYO Control - N = 1 Aldosterone in human serum Preservative: NaN ₃ (<0.1%)	1 Vial Lyophilized	Add 2 mL distilled water

1. **Calibrators:** After reconstitution, aliquot and freeze unused calibrators and control at -20°C. For the exact value, refer to the QC datasheet.
2. **Control:** Reconstitute with 2 ml of distilled water. After reconstitution, aliquot and freeze unused control at -20°C. For the exact value, refer to the QC datasheet.
3. **Coated Tubes:** Unused tubes must be stored at 2-8°C , protected from moisture.

3. MATERIAL REQUIRED BUT NOT SUPPLIED

- Plastic test tubes.
- Test tube racks.
- Adjustable, automatic micropipettes with disposable tips.
- Vortex mixer.
- Graduated cylinder.
- Aspiration pump or automated washing device.
- Scintillation gamma counter.
- Distilled water.
- Orbital shaker adjustable at 150 rpm.
- Hydrochloric acid.

4. WARNINGS AND PRECAUTIONS FOR USERS

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

Only experienced laboratory personnel should use this test and handling should be in agreement with GLP.

Radioactive Material - Not for Internal or External Use in Humans or Animals.

This radioactive material may be received, acquired, possessed, and used only by physicians, laboratories or hospitals and only for laboratory tests not involving internal or external administration of the material, or the radiation there from to human beings or animals. Its receipt, acquisition, possession, use and transfer are subject to the regulations of each country.

Physical characteristics of ¹²⁵I: see end of instructions

1. Safety Precautions: The following precautions should be observed in handling radioactive material:

- **Store radioactive materials in a designated area.**
- **Do not eat, drink, smoke or apply cosmetics where radioactive materials are being handled.**
- **Do not pipette by mouth.**
- **Wear gloves when handling radioactive materials and wash hands thoroughly afterwards.**
- **Cover working area with disposable absorbent paper.**
- **Wipe up all spills immediately and thoroughly and dispose of the contaminated materials as radioactive waste.**
- **Dispose of the liquid radioactive waste into the sanitary sewage system if permitted by the local regulations.**

2. CHEMICAL HAZARD Sodium Azide (NaN₃) Warning: Some of the reagents in this kit contain sodium azide as a preservative. For all such reagents, the concentration of sodium azide is <0.1% w/w. Sodium azide may react with lead and copper plumbing to form explosive metal azides. Dispose of all non radioactive reagents by flushing with large amounts of water through the plumbing system.

H370 : very toxic if swallowed

EUH 031: contact with acids liberates very toxic gas

3. POTENTIAL BIOHAZARDOUS MATERIAL Warning: This kit may contain some reagents made with human serum or plasma. The serum or plasma used has been tested by an FDA-approved method and found to be non-reactive for HIV-1/2 Antibodies, HCV and HBsAg. Because no method can offer complete assurance that HIV-1/2, HCV, HBsAg or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood samples in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories," 3rd Edition 1993.

For more information, see Material Safety Data Sheet (MSDS).

5. SAMPLE COLLECTION

The assay can be performed in serum or urine samples. Since Aldosterone shows a circadian rhythm we suggest to note down the sample collecting time. Highly lipemic or hemolyzed samples must be discarded. Keep samples at 2-8°C for 1-2 days; for longer periods it is advisable to freeze samples at -20°C. Repeated freezing and thawing of samples should be avoided.

Urine SamplesUrine collection.

Collect 24-hour urine by adding boric acid (10 g/l) as preservative. Record the volume and store at 2-8°C for 1-2 days; freeze at -20°C for longer time periods.

Urine Hydrolysis:

1. Prepare uncoated tubes, one for each urine sample.
2. Pipette 100 µl of urine sample.
3. Add 1 ml of 0.1 M hydrochloric acid into each tube.
4. Incubate for 15-20 hours at 30°C.
5. Use 50 µl of this solution for the test.

6. ASSAY PROCEDURE

- Bring all reagents and samples to room temperature (18-25°C) prior to use.
 - Before use, mix the samples by inversion.
 - For all calibrators, a duplicated measure is recommended.
 - Each tube can only be used once.
1. Prepare plain tubes for Total Activity (T) as well as coated tubes for Zero Calibrator (Bo), Calibrators (1-6), Control and Samples.
 2. Pipette **200 µl** of each Calibrator, Control and Sample into each tube.
 3. **Urine Samples.** Pipette **50 µl** of Hydrolyzed sample into each tube and add **150 µl** of the Zero Calibrator.
 4. Add **500 µl** of Radioactive Tracer into all tubes.
 5. Mix test-tube rack manually. Do not use vortex.
 6. Incubate: **Procedure A:** 18-24 hours at room temperature (18-25°C); or
Procedure B: 3hours at room temperature (18-25°C) with an orbital shaker set at 150 rpm.
 7. Carefully aspirate the incubation mixture from all tubes, except those for total activity, with an aspiration pump or decant by drying the edges of the tubes with blot-paper.
 8. Count the radioactivity in the tubes for 1 minute by using a gamma counter. We suggest to check the background of the instrument before counting the assay. In order to avoid variations in the sensitivity of the system, the background should be reduced to a minimum or adjusted properly.

ASSAY SCHEME

Tubes	Total Activity	Cali-brators	Control	Samples	Urine samples
Reagent					
Calibrator 0	----	----	----	----	150 µl
Calibrators	----	200 µl	----	----	----
Controls	----	----	200 µl	----	----
Samples	----	----	----	200 µl	50 µl
Tracer	500 µl	500 µl	500 µl	500 µl	500 µl
- Incubate: 18 – 24 h R.T (18-25°C). or 3 h R.T. (18-25°C) shaking (150 rpm)					
- Aspirate					
- Count					

7. CALCULATION OF RESULTS

Draw the calibration curve with the calibration concentrations on the x-axis and the respective B/Bo % on the y-axis.

Calculate the B/Bo% for each sample and read the concentration by interpolating on the calibration curve to obtain the Aldosterone concentration in the tested samples, expressed in pg/ml.

Urine Samples

The concentration read on the calibration curve must be multiplied by factor 44 to obtain the Aldosterone concentration in pg/ml. In order to express Aldosterone concentration in $\mu\text{g}/24\text{h}$:

$$\mu\text{g}/24\text{h} = \frac{\text{Concentration (pg/ml)} \times 44 \times \text{Urine excreted (liters)}}{1000}$$

EXAMPLE OF CALCULATION

The values reported below must be considered as an example and may not be used in place of experimental data.

Description	Average cpm	B/B0 (%)	Aldosterone (pg/ml)
Total activity (T)	41999	-	-
CAL 0	19213	100	0
CAL 1	16439	85.4	25
CAL 2	14667	76.1	50
CAL 3	12268	63.6	100
CAL 4	8184	42.1	300
CAL 5	5844	29.9	600
CAL 6	3669	18.4	1500
CONTROL	11263	58.3	133
P1	14905	77.4	45.5
P2	9869	51.0	191
P3	7934	40.8	323

8. REFERENCE VALUES

It is recommended that each laboratory determines its own reference interval. Values reported below are only indicative.

Serum Aldosterone:	
- At rest:	10 - 160 pg/ml
- In motion:	35 - 300 pg/ml

8.1 Other sample type:

Urinary Aldosterone:	<18 $\mu\text{g}/24\text{h}$ (95th percentile)
Plasma EDTA :	<122 pg/ml (95th percentile)

9. PERFORMANCES OF THE ASSAY**SPECIFICITY**

The percentage of cross-reactivity is calculated by using the EP7-A2 method.

% Cross reactivity = $100 * ((\text{measured value} - \text{true value} / \text{interfering concentration}))$.

The present method has shown the following cross-reactions:

Substance	% cross reactivity
Cortisol	0.00
17 Hydroxyprogesterone	0.33
Progesterone	0.06
Androstenedione	0.07
Prednisolone	0.001
Corticosterone	0.015
11-Deoxycortisol	0.380
11-Deoxycorticosterone	7.952
Prednisone	0.001
Testosterone	0.238

SENSITIVITY**Analytical sensitivity**

The sensitivity was calculated based upon the calibration curve and expressed as the minimal dose showing a significant difference from the Zero Calibrator (mean value - 2 S.D.). This dose is 1.4 pg/ml.

Functional sensitivity

The functional assay sensitivity is the lowest value which is measured with a precision of maximum 20% inter-assay variance. For the Aldosterone, this value is 25 pg/ml.

PRECISION

Precision was evaluated upon intra- and inter-assay variability at different analyte concentrations.

Intra-assay

Serum	Mean	±	S.D.	C.V.	N
	(pg/ml)			(%)	
1	42.3	±	5.8	13.7	20
2	177	±	6.7	3.8	20
3	278	±	14.8	5.3	20

Inter-assay

Serum	Mean	±	S.D.	C.V.	N
	(pg/ml)			(%)	
1	38.2	±	7.1	18.6	9
2	169	±	12.6	7.5	9
3	284	±	17.7	6.2	9

ACCURACY

Accuracy of the method has been checked by the recovery and parallelism tests.

Recovery Test

Samples, mixed with equal volumes of each calibrator, were tested.

Added	Expected (pg/ml)	Measured (pg/ml)	Recovery (%)
S1	-	97.2	-
S1 + CAL 0	48.6	41.6	85.6
S1 + CAL 1	61.1	69.4	113.6
S1 + CAL 2	73.6	88.8	120.7
S1 + CAL 3	98.6	121	122.7
S1 + CAL 4	199	249	125.1
S1 + CAL 5	349	455	130.4
S1 + CAL 6	799	933	116.8
S2	-	142	-
S2 + CAL 0	71.0	68.4	96.3
S2 + CAL 1	83.5	83.4	99.9
S2 + CAL 2	96.0	96.9	100.9
S2 + CAL 3	121	137	113.2
S2 + CAL 4	221	244	110.4
S2 + CAL 5	371	431	116.2
S2 + CAL 6	821	952	116.0

Parallelism Test

Serums with high analyte concentration was tested at different dilutions with the Zero Calibrator.

Dilution	Expected (pg/ml)	Measured (pg/ml)	Recovery (%)
S1 undiluted	-	128	-
1/2	64.1	61.6	96.1
1/4	32.1	30.9	96.3
1/8	16.0	14.9	93.1
S2 undiluted	-	351	-
1/2	176	161	91.5
1/4	87.8	75	85.4
1/8	43.9	36.3	82.7
1/16	22.0	25	113.6














Physical characteristics of ¹²⁵I

$t_{1/2} = 59.9$ days

Main emissions

	E (MeV)	%
γ	0.035	
X	0.027	114
	0.032	25

SYMBOLS USED WITH IBL-AMERICA ASSAYS

Symbol	English	Deutsch	Française	Espanol	Italiano
	European Conformity	CE-Konformitäts-kennzeichnung	Conforme aux normes européennes	Conformidad europea	Conformità europea
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las Instrucciones	Consultare le istruzioni per l'uso
	In vitro diagnostic device	In-vitro-Diagnostikum	utilisation Diagnostic in vitro	Diagnóstico in vitro	Per uso Diagnostica in vitro
	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
	Catalogue number	Katalog-Nr.	Référence	Número de catálogo	No. di catalogo
	Lot. No. / Batch code	Chargen-Nr.	No. de lot	Número de lote	Lotto no
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Note warnings and precautions	Warnhinweise und Vorsichtsmaßnahmen beachten	Avertissements et mesures de précaution font attention	Tiene en cuenta advertencias y precauciones	Annoti avvisi e le precauzioni
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservacion	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeitsdatum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
<i>Distributed by</i>	Distributed by	Vertrieb durch	Distribution par	Distribución por	Distribuzione da parte di
	Version	Version	Version	Versión	Versione
	Single-use	Einmalverwendung	À usage unique	Uso único	Uso una volta