



ALDOSTERONE KITS

Part # 64ALDPEG & 64ALDPEH

Test size#: 500 tests (64ALDPEG) and 10,000 tests (64ALDPEH) - assay volume: 20 μ L

Revision: #05 of September 2023

Store at: -60°C or below (64ALDPEG); -60°C or below (64ALDPEH)

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

ASSAY PRINCIPLE

This kit is intended for the simple and rapid quantification of Aldosterone and offers a fast alternative to ELISA.

The detection principle of this kit is based on HTRF® technology (Homogeneous Time-Resolved Fluorescence). As shown in Figure 1, Aldosterone is detected in a competitive assay by using anti Aldosterone antibody labeled with Europium cryptate (donor), and Aldosterone labeled with XL665 (acceptor).

When the dyes are in close proximity, the excitation of the donor with a light source (laser or flash lamp) triggers a Fluorescence Resonance Energy Transfer (FRET) towards the acceptor, which in turn fluoresces at a specific wavelength (665 nm). The Aldosterone present in the sample competes with the binding between the two HTRF detection solutions and thereby prevents FRET from occurring. The specific signal is inversely proportional to the Aldosterone concentration.

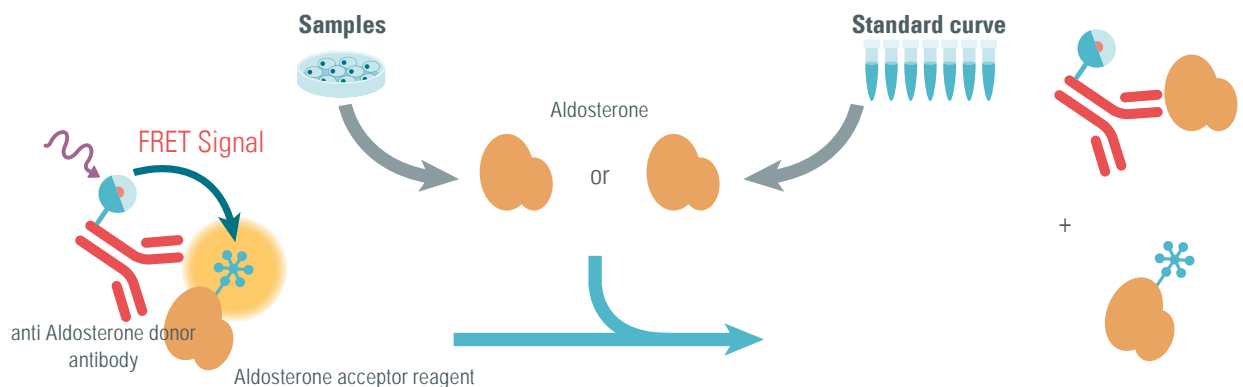
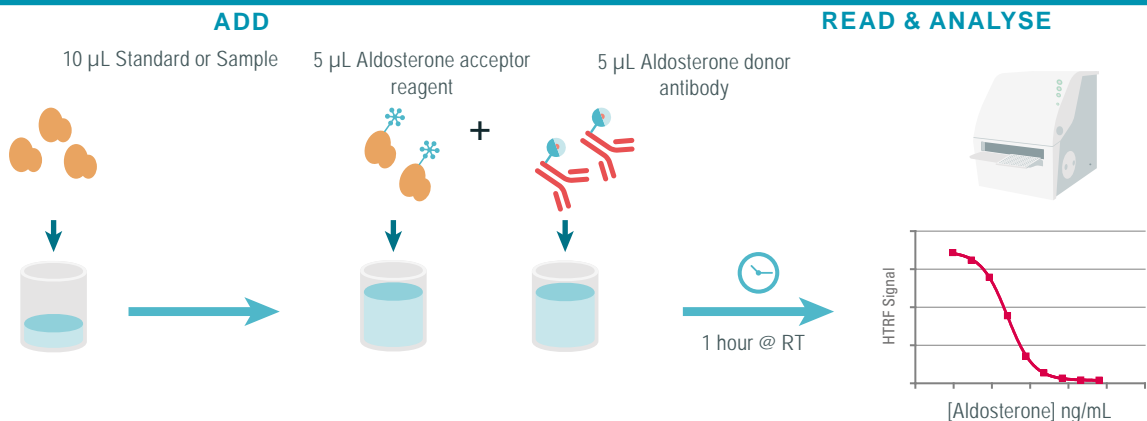


Figure 1: Principle of HTRF Aldosterone competitive assay.

MANUAL AT A GLANCE



Do not pre-mix the XL665 and Cryptate solutions prior to dispensing.

Make sure to use the set-up for Eu Cryptate.

MATERIALS PROVIDED:

KIT COMPONENTS	500 TESTS * CAT # 64ALDPEG	10,000 TESTS * CAT # 64ALDPEH
Aldosterone Standard Frozen	1 vial - 50 µL 100 µg/mL - DMSO	1 vial - 50 µL 100 µg/mL - DMSO
anti Aldosterone antibody Eu Cryptate antibody	1 vial - 50 µL Frozen - 50X	1 vial - 1 mL Frozen - 50X
Aldosterone XL665 reagent	1 vial - 50 µL Frozen - 50X	1 vial - 1 mL Frozen - 50X
Diluent ** ready-to-use	1 vial 20 mL	1 vial 20 mL
Detection buffer *** ready to use	1 vial 7 mL	1 vial 105 mL

* When used as advised, the two available kit sizes will provide sufficient reagents for 500 tests and 10,000 tests respectively in 20 µL final volume..

Assay volumes can be adjusted proportionally to run the assay in 96 or 1536 well microplates.

** Medium like cell culture medium can be an alternative to the diluent.

*** The Detection buffer is used to prepare working solutions of acceptor and donor reagents.

PURCHASE SEPARATELY:

- HTRF®-Certified Reader. **Make sure the setup for Eu Cryptate is used.**

For a list of HTRF-compatible readers and set-up recommendations, please visit www.revvy.com

- Small volume (SV) detection microplates - .

For more information about microplate recommendations, please visit our website at: www.revvy.com

STORAGE AND STABILITY

Store the kit at -60°C or below.

Under proper storage conditions, reagents are stable until the expiry date indicated on the label. Diluent and detection buffer are shipped frozen, but can be stored at 2-8°C in your premises.






If lyophilized, reconstituted reagents, antibodies, and standard stock solutions may be frozen and thawed only once. To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at -60°C or below.

REAGENT PREPARATION**BEFORE YOU BEGIN:**

- It is very important to prepare reagents in the specified buffers. The use of an incorrect diluent may affect reagent stability and assay results.
- Before use, allow Diluent and Detection buffer to warm up at room temperature and homogenize them with a vortex.
- Thaw all reagents at room temperature, allow them to warm up.
- Antibody solutions must be prepared in individual vials and can be mixed prior to dispensing.
- Aldosterone standards (for standard curve) must be prepared in diluent or in the same medium as the samples.

TAKE CARE TO PREPARE STOCK AND WORKING SOLUTIONS ACCORDING TO THE DIRECTIONS FOR THE KIT SIZE YOU HAVE PURCHASED.

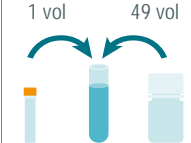
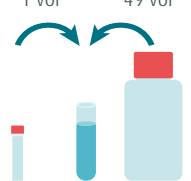
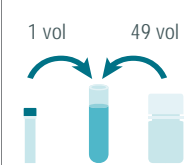
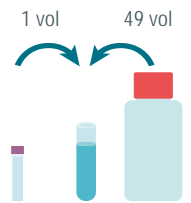
TO PREPARE REAGENT STOCK SOLUTIONS:

500 TESTS KIT - 64ALDPEG		10,000 TESTS KIT - 64ALDPEH	
anti Aldosterone antibody Eu Cryptate antibody			
Thaw the anti Aldosterone antibody Eu Cryptate antibody. Mix gently. This 50X stock solution can be frozen and stored at -60°C or below.			Thaw the anti Aldosterone antibody Eu Cryptate antibody. Mix gently. This 50X stock solution can be frozen and stored at -60°C or below.
Aldosterone XL665 reagent			
Thaw the Aldosterone XL665 reagent. Mix gently. This 50X stock solution can be frozen and stored at -60°C or below.			Thaw the Aldosterone XL665 reagent. Mix gently. This 50X stock solution can be frozen and stored at -60°C or below.
Aldosterone Standard			
Thaw the Aldosterone standard solution in order to obtain a 100 µg/mL (see vial label) stock solution. Mix gently.			Thaw the Aldosterone standard solution in order to obtain a 100 µg/mL (see vial label) stock solution. Mix gently.
Diluent			
The diluent is ready-to-use			The diluent is ready-to-use
Detection buffer			
The Detection buffer is ready-to-use.			The Detection buffer is ready-to-use.

TO PREPARE ANTIBODY WORKING SOLUTIONS:

Each well requires 5 µL anti Aldosterone antibody Eu Cryptate antibody and 5 µL Aldosterone XL665 reagent.

Prepare the two solutions in separate vials.

500 TESTS KIT - 64ALDPEG		10,000 TESTS KIT - 64ALDPEH	
anti Aldosterone antibody Eu Cryptate antibody			
Dilute 50-fold the stock solution of Aldosterone Cryptate antibody with detection buffer#3 e.g. take 0.05 mL of Eu Cryptate antibody stock solution and add it to 2.45 mL of detection buffer #3.			Dilute 50-fold the stock solution of Aldosterone Eu Cryptate antibody with detection buffer#3 e.g. take 1 mL of Cryptate antibody stock solution and add it to 49 mL of detection buffer #3.
Aldosterone XL665 reagent			
Dilute 50-fold the stock solution of Aldosterone XL665 reagent with detection buffer: e.g. take 0.05 mL of XL665 reagent stock solution and add it to 2.45 mL of detection buffer #3.			Dilute 50-fold the stock solution of Aldosterone XL665 reagent with detection buffer: e.g. take 1 mL of XL665 reagent stock solution and add it to 49 mL of detection buffer #3.
Antibody mix			
Do not pre-mix the XL665 and the Eu Cryptate solutions prior to dispensing.			

TO PREPARE STANDARD WORKING SOLUTIONS:

- Each well requires 10 μL of standard.
- Dilute the standard stock solution serially with diluent or in the medium used for the preparation of the samples.
- In order to check for a potential interference effect from your own assay buffer when using the assay for the first time, we highly recommend the parallel preparation of a standard curve in your own supplemented cell culture medium and in diluent.
- In order to counteract any standard sticking, we recommend changing tips between each dilution.

A recommended standard dilution procedure is listed and illustrated below:

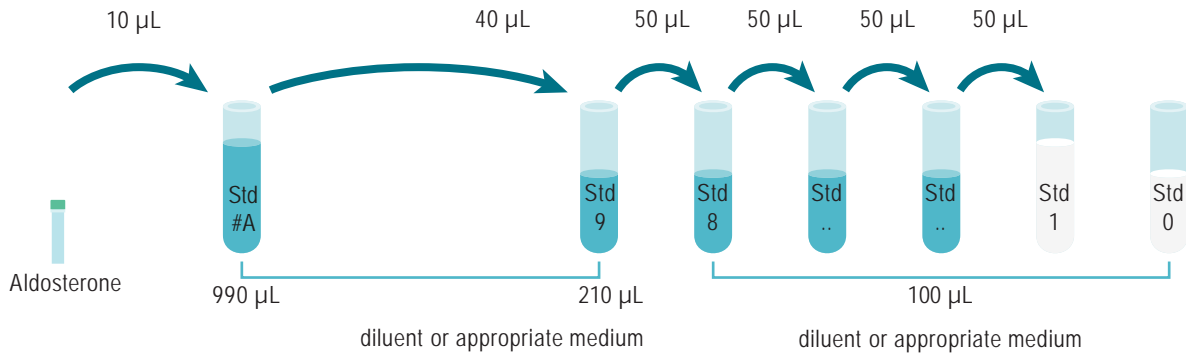
Dilute the standard stock solution 100-fold with diluent; this yields the Intermediate Standard Solution # A (1000 ng/mL). e.g: take 10 μL of standard stock solution and add it to 990 μL of diluent. Mix gently.

Dilute the intermediate Standard Solution #A 6.25-fold with diluent to prepare high standard (Std 9): e.g. take 40 μL of intermediate Standard Solution #A and add it to 210 μL of diluent. Mix gently.

Use the high standard (Std 9) to prepare the standard curve using 1/3 serial dilutions as follows:

- Dispense 100 μL of diluent in each vial from Std 8 to Std 0.
- Add 50 μL of standard to 100 μL of diluent, mix gently and repeat the 1/3 serial dilution to make standard solutions: std8, std7, std6, std5, std4, std3, std2, std1.

This will create 9 standards for the analyte. Std 0 (Positive control) is diluent or appropriate culture medium alone.








STANDARD	SERIAL DILUTIONS	ALDOSTERONE WORKING SOLUTION (ng/mL)
Standard Stock solution	Thawed stock solution	100,000
Intermediate standard solution #A	10 μL Standard stock solution + 990 μL Diluent	1,000
Standard 9	40 μL Intermediate Standard Solution #A + 210 μL Diluent	160
Standard 8	50 μL standard 9 + 100 μL Diluent	53.3
Standard 7	50 μL standard 8 + 100 μL Diluent	17.7
Standard 6	50 μL standard 7 + 100 μL Diluent	5.9
Standard 5	50 μL standard 6 + 100 μL Diluent	2
Standard 4	50 μL standard 5 + 100 μL Diluent	0.65
Standard 3	50 μL standard 4 + 100 μL Diluent	0.22
Standard 2	50 μL standard 3 + 100 μL Diluent	0.07
Standard 1	50 μL standard 2 + 100 μL Diluent	0.02
Standard 0	100 μL Diluent	0

TO PREPARE SAMPLES:

- Each well requires 10 μ L of sample.
- Just after their collection, put the samples at 4°C and test them immediately. For later use, samples should be dispensed into disposable plastic vials and stored at -60°C or below. Avoid multiple freeze/thaw cycles.
- Samples with a concentration above the highest standard (Std 9) must be diluted diluent or in your appropriate sample medium.

ASSAY MANUAL

		Negative control or Cryptate control	Standard (Std 0 - Std 9)	Samples
Step 1 		Dispense 10 μ L of diluent into each negative control well	Dispense 10 μ L of each Aldosterone standard (Std 0 - Std 9) into each standard well	Dispense 10 μ L of each sample into each sample well
Step 2 		Add 5 μ L of detection buffer to all negative control wells	Add 5 μ L Aldosterone acceptor reagent working solution to all wells	
Step 3 		Add 5 μ L Aldosterone donor antibody working solution to all wells		
Step 4 		Seal the plate and incubate 1 hour @ RT		
Step 5 		Remove the plate sealer and read on an HTRF® compatible reader		

DATA REDUCTION

1. Calculate the ratio of the acceptor and donor emission signals for each individual well.

$$\text{Ratio} = \frac{\text{Signal 665 nm}}{\text{Signal 620 nm}} \times 10^4$$

2. Calculate the % CVs. The mean and standard deviation can then be worked out from ratio replicates.

$$\text{CV (\%)} = \frac{\text{Standard deviation}}{\text{Mean Ratio}} \times 100$$

3. Calculate the % delta F which reflects the signal to background of the assay. The negative control plays the role of an internal assay control. Delta F is used for the comparison of day to day runs of the same assay.

$$\text{delta F (\%)} = \frac{\text{Ratio Standard or sample} - \text{Ratio Negative Control}}{\text{Ratio Negative Control}} \times 100$$

For more information about data reduction, please visit www.revivity.com

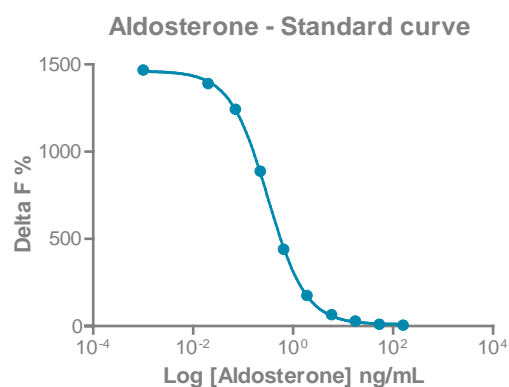
RESULTS

This data must not be substituted for the data obtained in the laboratory and should be considered only as an example.

Results may vary from one HTRF® compatible reader to another.

The assay standard curve is created by plotting delta F% versus the analyte concentration.

	Ratio ⁽¹⁾	CV ⁽²⁾	Delta F% ⁽³⁾
Negative control	459	3.8%	
Std 0 – Positive control	7,190	2.2%	1,468%
Std 1 - 0.02 ng/mL	6,835	1.4%	1,390%
Std 2 - 0.07 ng/mL	6,159	1.9%	1,243%
Std 3 - 0.22 ng/mL	4,532	2.3%	888%
Std 4 - 0.65 ng/mL	2,480	2.8%	441%
Std 5 - 1.9 ng/mL	1,260	3.3%	175%
Std 6 - 5.9 ng/mL	758	3.8%	65%
Std 7 - 17.7 ng/mL	591	4.2%	29%
Std 8 - 53.3 ng/mL	503	4.7%	10%
Std 9 - 160 ng/mL	479	5.2%	5%



ANALYTICAL CHARACTERISTICS

CROSS-REACTIVITY

	Cross-reactivity (%)		Cross-reactivity (%)		Cross-reactivity (%)
Aldosterone	100.00%	Cortisol	0.00%	Prednisolone	0.00%
3 beta, 5 alpha tetrahydroaldosterone	7.60%	11-deoxycortisol	0.07%	Prednisone	0.00%
3 alpha, 5 beta tetrahydroaldosterone	0.09%	Cortisone	0.01%	Pregnenolone	0.00%
Androsterone	0.00%	Dexamethasone	0.00%	Pregnanetriol	0.00%
DHEA	0.00%	Estradiol	0.00%	Testosterone	0.01%
Androstendione	0.00%	Estriol	0.00%	Canrenone	0.00%
Cortisolone	0.01%	Estrone	0.00%	Prazosin-HCl	0.00%
Corticosterone	0.05%	9 alphafludrocortisone	0.00%	Spironolactone	0.00%
18-hydroxydeoxycorticosterone	0.01%	Progesterone	0.00%		
11-deoxycorticosterone	0.00%	17 alpha-hydroxyprogesterone	0.01%		

DETECTION LIMIT & EC50 WORKING CONCENTRATIONS

Detection limit	25 pg/mL
EC50	300 pg/mL



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