MANUAL

AlphaLISA® Corticosterone Detection Kit

Product number: AL3020 HV/C/F

Research Use Only. Not for use in diagnostic procedures.

Product Information

Application: This kit is designed for the quantitative determination of Corticosterone in serum and plasma of mammalian species and chickens using a homogeneous AlphaLISA assay (no wash steps). The assay shows mimimun cross reactivity with other steroids (see page 9).

Sensitivity: Lower Detection Limit (LDL): 9 ng/mL

IC_{50}: 37 ng/mL

Dynamic range: 0.02 – 8000 ng/mL (Figure 1).

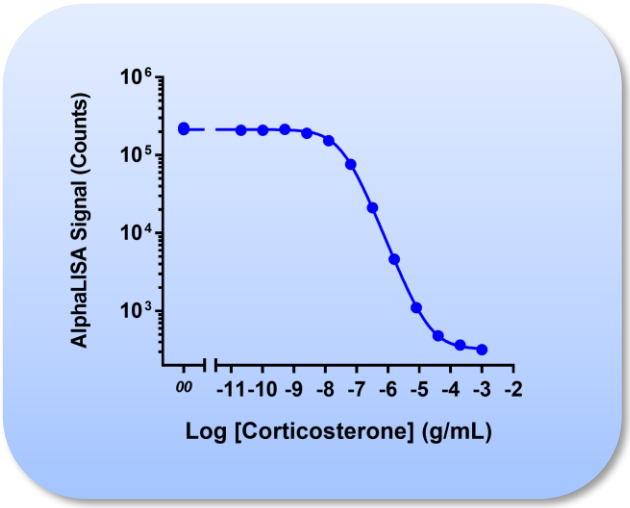


Figure1. Typical sensitivity curves in NaCl Buffer. The data was generated using a white Optiplate™ 384 microplate and the EnVision® Multilabel Plate Reader 2103 with Alpha option.

Storage: Store kit in the dark at +4˚C except the analyte that should be stored at room tempertature.

Stability: This kit is stable for at least 12 months from the manufacturing date when stored in its original packaging and the recommended storage conditions.

Analyte of Interest

Corticosterone (CORT) is a member of corticosteroids and is also known as 17-deoxycortisol or 11β 21-dihydroprogesterone. CORT is produced in response to the stimulation of the adrenal cortex by adrenocorticotropic hormone and is the precursor of pregnenolone, cortisol, and aldosterone. CORT production has been shown to increase with stress and the elevated CORT levels have been associated with impairment of long term memory retrieval. In some species, CORT is a main glucocorticoid, involved in regulation of energy, immune reactions, and stress responses. Furthermore, CORT serves as a major homeostatic modulator of sodium and potassium levels in vivo.

Description of the AlphaLISA Assay

AlphaLISA technology allows the detection of molecules of interest in buffer and in serum and plasma in a highly sensitive, quantitative, reproducible and user-friendly mode. Biotinylated corticosterone-BSA complex and free corticosterone (in standards or in samples) compete for binding to anti-corticosterone antibody that is conjugated to AlphaLISA Acceptor beads. In the presence of Streptavidin-coated Alpha Donor beads, biotinylated Corticosterone-BSA complex binds to the Streptavidin-coated Alpha Donor beads. When corticosterone-BSA binds to anti-corticosterone AlphaLISA acceptor beads, the beads come into close proximity. The excitation of the Donor beads provokes the release of singlet oxygen molecules that triggers a cascade of energy transfer in the Acceptor beads, resulting in a sharp peak of light emission at 615 nm (Figure 2).

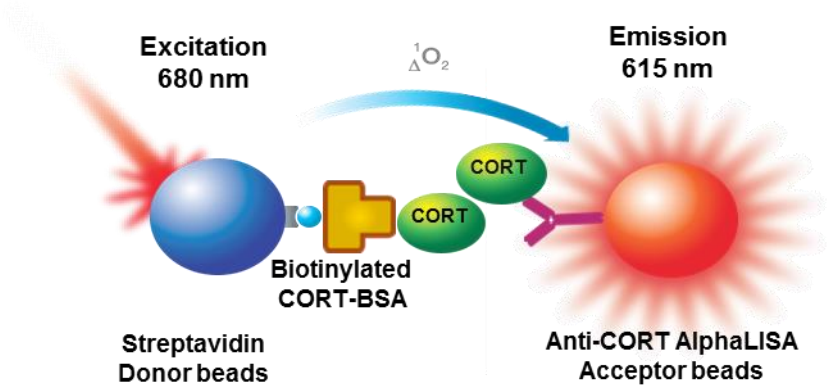


Figure 2. AlphaLISA Assay Principle.

Precautions

- The Alpha Donor beads are light-sensitive. All the other assay reagents can be used under normal light conditions. All Alpha assays using the Donor beads should be performed under subdued laboratory lighting (< 100 lux). Green filters (LEE 090 filters (preferred) or Roscolux filters #389 from Rosco) can be applied to light fixtures.
- All blood components and biological materials should be handled as potentially hazardous.
- Some analytes are present in blood. Take precautionary measures to avoid contamination of the reagent solutions.
- The Biotinylated Anti-Analyte Antibody contains sodium azide. Contact with skin or inhalation should be avoided.

Kit Contents

Kit components	AL3020HV (100 assay points***)	AL3020C (500 assay points***)	AL3020F (5000 assay points***)
AlphaLISA Anti-CORT Acceptor beads stored in PBS, 0.05% Kathon, pH 7.2	20 µL @ 5 mg/mL (1 brown tube, white cap)	50 µL @ 5 mg/mL (1 brown tube, white cap)	500 µL @ 5 mg/mL (1 brown tube, white cap)
Streptavidin (SA)-coated Donor beads stored in 25 mM HEPES, 100 mM NaCl, 0.05% Kathon, pH 7.4	40 µL @ 5 mg/mL (1 brown tube, black cap)	100 µL @ 5 mg/mL (1 brown tube, black cap)	1 mL @ 5 mg/mL (1 brown tube, black cap)
Biotinylated CORT-BSA Tracer stored in PBS, 0.1% Tween-20, 0.05% NaN_3, pH 7.4	20 µL @ 500 nM (1 tube, black cap)	50 µL @ 500 nM (1 tube, black cap)	500 □L µL @ 500 nM (1 tube, black cap)
CORT Analyte In DMSO*	100 µL @ 10 mg/mL (1 tube, clear cap)	100 µL @ 10 mg/mL (1 tube, clear cap)	100 µL @ 100 mg/mL (1 tube, clear cap)
NaCl Buffer (5X) **	2 mL, 1 small bottle	10 mL, 1 small bottle	100 mL, 1 large bottle

* It has been demonstrated that CORT DMSO solution is stable for at least 6 months at room temperature. One vial contains an amount of CORT sufficient for performing 8 standard curves. Additional vials can be ordered separately (cat # AL3020S).

** Contains 125 mM HEPES, pH 7.4, 2.5 M NaCl, 5 mg/mL Dextran-500, 2.5% Triton X-100, 0.25% Kathon, 2.5%. Extra buffer can be ordered separately (cat # AL007C: 10 mL, cat # AL007F: 100 mL).

*** The number of assay points is based on an assay volume of 100 µL in 96-well plates or 50 µL in 384-well assay plates using the kit components at the recommended concentrations.

Sodium azide should **not** be added to the stock reagents. High concentrations of sodium azide (> 0.001 % final in the assay) might decrease the AlphaLISA signal.

Specific additional required reagents and materials:

The following materials are recommended:

Item	Suggested source	Catalog #
TopSeal™-A Plus Adhesive Sealing Film	Revvity Inc.	6050185
EnVision®-Alpha Reader	Revvity Inc.	-

Recommendations

- The volume indicated on each tube is guaranteed for single pipetting. Multiple pipetting of the reagents may reduce the theoretical amount left in the tube. To minimize loss when pipetting beads, it is preferable not to pre-wet the tip.
- Centrifuge all tubes (including lyophilized analyte) before use to improve recovery of content (2000g, 10-15 sec). Re-suspend all reagents by vortexing before use.

- Use Milli-Q® grade H_2O (18 MΩ•cm) to dilute 5X NaCl Buffer.
- When diluting the standard or samples, change tips between each standard or sample dilution. When loading reagents in the assay microplate, change tips between each standard or sample addition and after each set of reagents.
- When reagents are added to the microplate, make sure the liquids are at the bottom of the well.
- Small volumes may be prone to evaporation. It is recommended to cover microplates with TopSeal-A Plus Adhesive Sealing Films to reduce evaporation during incubation. Microplates can be read with the TopSeal-A Plus Film.
- The AlphaLISA signal is detected with an EnVision Multilabel Reader equipped with the Alpha option using the AlphaScreen standard settings (e.g. Total Measurement Time: 550 ms, Laser 680 nm Excitation Time: 180 ms, Mirror: D640as, Emission Filter: M570w, Center Wavelength 570 nm, Bandwidth 100 nm, Transmittance 75%).
- AlphaLISA signal will vary with temperature and incubation time. For consistent results, identical incubation times and temperature should be used for each plate.
- The standard curves shown in this technical data sheet are provided for information only. A standard curve must be generated for each experiment. The standard curve should be performed in the fetal bovine serum for serum and/or plasma samples.

Assay Procedure

IMPORTANT: PLEASE READ THE RECOMMENDATIONS BELOW BEFORE USE

- The protocol described below is an example for generating one standard curve in a 50 µL final assay volume (48 wells, triplicate determinations). The protocols also include testing samples in 452 wells. If a different amount of samples are tested, the volumes of all reagents have to be adjusted accordingly, as shown in the table below. These calculations do not include excess reagent to account for losses during transfer of solutions or dead volumes.
- The standard dilution protocol is provided for information only. As needed, the number of replicates or the range of concentrations covered can be modified.
- Use of four background points in triplicate (12 wells) is recommended when LDL is calculated. One background point in triplicate (3 wells) can be used when LDL is not calculated.

		Volume					
Format	**# of data points**	**Final**	**Sample**	**AlphaLISA beads**	**Biotin CORT-BSA Tracer**	**Donor beads**	**Plate recommendation**
AL3020HV	100	100 µL	40 µL	20 µL	20 µL	20 µL	White OptiPlate-96 (cat # 6005290) White ½ AreaPlate-96 (cat # 6005560)
AL3020C	250	100 µL	40 µL	20 µL	20 µL	20 µL	White OptiPlate-96 (cat # 6005290) White ½ AreaPlate-96 (cat # 6005560)
	500	50 µL	20 µL	10 µL	10 µL	10 µL	White ½ AreaPlate-96 (cat # 6005560) White OptiPlate-384 (cat # 6007290) Light gray AlphaPlate™-384 (cat # 6005350)
	1 250	20 µL	8 µL	4 µL	4 µL	4 µL	Light gray AlphaPlate-384 (cat # 6005350) ProxiPlate™-384 Plus (cat # 6008280) White OptiPlate-384 (cat # 6007290)
	2 500	10 µL	4 µL	2 µL	2 µL	2 µL	Light gray AlphaPlate-1536 (cat # 6004350)
AL3020F	5 000	50 µL	20 µL	10 µL	10 µL	10 µL	White ½ AreaPlate-96 (cat # 6005560) White OptiPlate-384 (cat # 6007290) Light gray AlphaPlate-384 (cat # 6005350)
	12 500	20 µL	8 µL	4 µL	4 µL	4 µL	Light gray AlphaPlate-384 (cat # 6005350) ProxiPlate-384 Plus (cat # 6008280) White OptiPlate-384 (cat # 6007290)
	25 000	10 µL	4µL	2 µL	2 µL	2 µL	Light gray AlphaPlate-1536 (cat # 6004350)

The protocol (3 incubation steps) described below is for 500 assay points including one standard curve (48 wells) and samples (452 wells). *If a different amount of samples are tested, the volumes of all reagents have to be adjusted accordingly.*

1) Preparation of 1X NaCl Buffer:

Add 10 mL of 5X NaCl Buffer to 40 mL H_2O. **Ensure the buffer is completely solubilized (may require heating and stirring)**

2) Preparation of CORT analyte standard dilutions:

a. Analyte is provided as 100 µL at 10 mg/mL in DMSO solution, use directly. If it is stored or shipped at 4ºC, warm the analyte solution to room temperature before using.
b. Prepare standard dilutions as follows in 1X NaCl Buffer (change tip between each standard dilution):

Tube	Vol. of CORT (µL)	Vol. of diluent (µL) *	[CORT] in standard curve	
			(g/mL in 5 µL)	(ng/mL in 5 µL)
A	12 µL of CORT solution	108	1.E-03	1 000 000
B	40 µL of tube A	160	2.E-04	200 000
C	40 µL of tube B	160	4.E-05	40 000
D	40 µL of tube C	160	8.E-06	8 000
E	40 µL of tube D	160	2.E-06	1 600
F	40 µL of tube E	160	3.E-07	320
G	40 µL of tube F	160	6.E-08	64
H	40 µL of tube G	160	1.E-08	12.8
I	40 µL of tube H	160	3.E-09	2.6
J	40 µL of tube I	160	5.E-10	0.5
K	40 µL of tube J	160	1.E-10	0.1
L	40 µL of tube K	160	2.E-11	0.02
M ** (background)	0	100	0	0
N ** (background)	0	100	0	0
O ** (background)	0	100	0	0
P ** (background)	0	100	0	0

* Dilute standards in diluent (e.g. 1X NaCl Buffer).
At low concentrations of analyte, a significant amount of analyte can bind to the vial. Therefore, load the analyte standard dilutions in the assay microplate within 60 minutes of preparation.

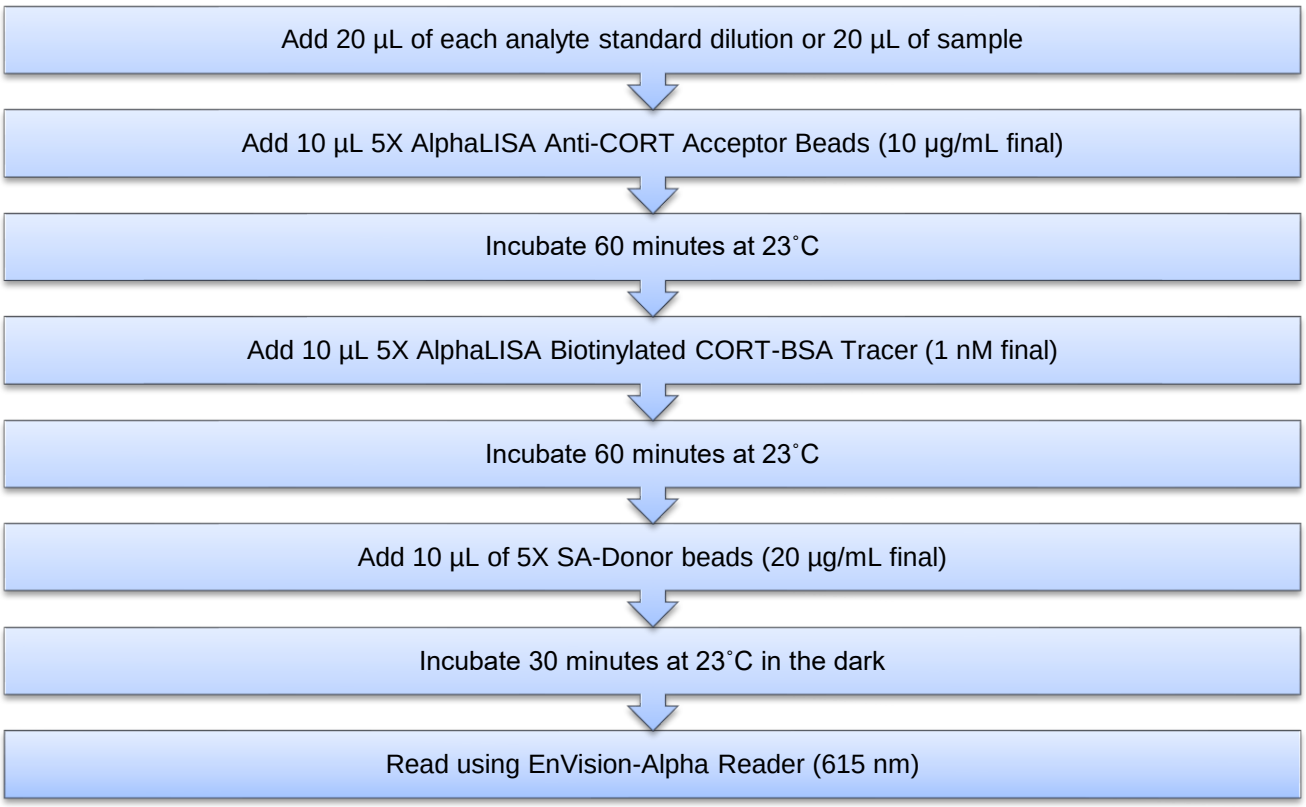
** Four background points in triplicate (12 wells) are used when LDL is calculated. If LDL does not need to be calculated, one background point in triplicate can be used (3 wells).

3) Preparation of 5X AlphaLISA Anti-CORT Acceptor beads (50 µg/mL):
a. Prepare just before use.
b. Add 50 µL of 5 mg/mL AlphaLISA Anti-CORT Acceptor beads to 4950 µL of 1X NaCl Buffer.

4) Preparation of 5X Biotinylated CORT-BSA Tracer (5 nM):
a. Prepare just before use.
b. Add 50 µL of 500 nM Biotinylated CORT-BSA Tracer to 4950 µL of 1X NaCl Buffer.

5) Preparation of 5X Streptavidin (SA) Donor beads (100 µg/mL):
a. Keep the beads under subdued laboratory lighting.
b. Prepare just before use
c. Add 100 µL of 5 mg/mL SA-Donor beads to 4900 µL of 1X NaCl Buffer.

6) In a white Optiplate (384 wells):



Data Analysis

- Calculate the average count value for the background wells.
- Generate a standard curve by plotting the AlphaLISA counts versus the concentration of analyte. A log scale can be used for either or both axes. No additional data transformation is required.
- Analyze data according to a nonlinear regression using the 4-parameter logistic equation (sigmoidal dose-response curve with variable slope) and a $1/Y^2$ data weighting (the values at maximal concentrations of analyte after the hook point should be removed for correct analysis).
- The LDL is calculated by interpolating the average background counts (12 wells without analyte) - 3 x standard deviation value (average background counts - (3xSD)) on the standard curve.
- Read from the standard curve the concentration of analyte contained in the samples.
- If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Assay Performance Characteristics

AlphaLISA assay performance described below was determined using the 3 step protocol performed in NaCl Buffer.

- Assay Sensitivity and Precision

The LDL was calculated as described above. The values correspond to the lowest concentration of analyte that can be detected in a volume of 5 µL using the recommended assay conditions. The results of 6 independent assays are shown. The intra-assay coefficient of variation (%CV) was determined using a total of 7 independent determinations in triplicate and the inter-assay coefficient of variation (%CV) was determined using a total of 7 independent determinations with 21 measurements.

Assay Sensitivity and Precisions	**NaCl Buffer**
LDL (ng/mL)	9
Intra assay CV%	3.2
Inter assay CV%	15.9

- Spike Recovery:

Three known concentrations of analyte were spiked into NaCl Buffer. The average recovery from three independent measurements is reported.

Spiked CORT (ng/mL)	**% Recovery in NaCl Buffer**
316	99
64	98
13	109

- Specificity:

Cross-reactivity of the CORT AlphaLISA Kit was tested using the following steroids at 20 µg/mL in NaCl Buffer.

Steroids	**% Cross-reactivity**
Aldosterone	2.8
Cortisol	0.2
Cortisone	0.0
Hydrocortisone	0.4
Estradiol	0.0
Progesterone	0.0

- Serum and Plasma tests:

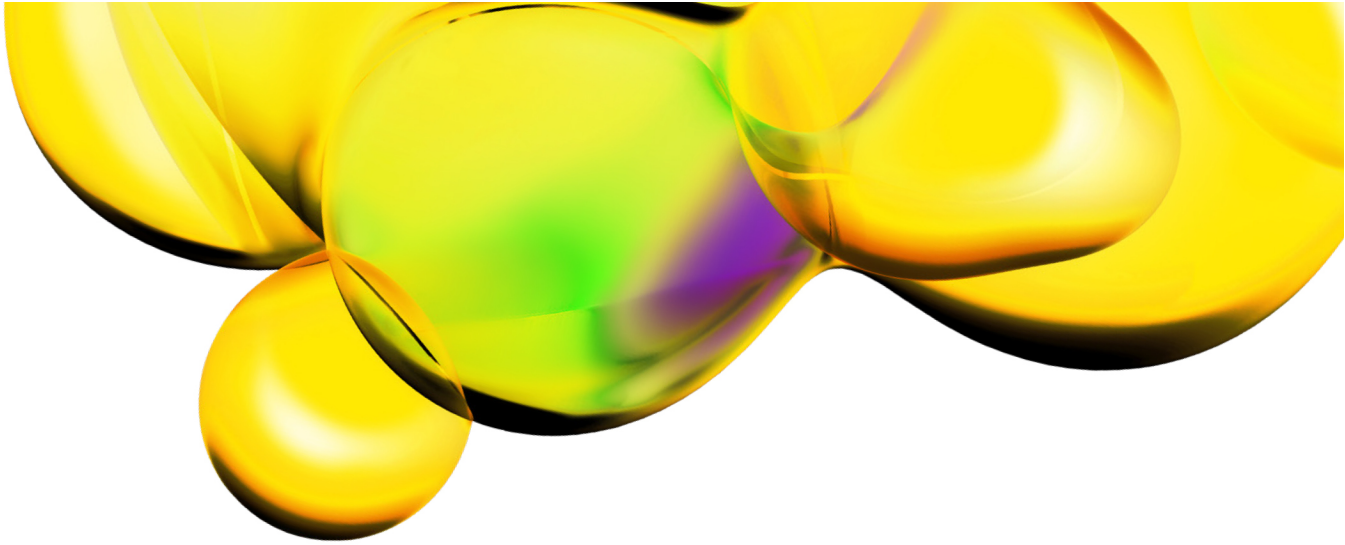
To validate the assay kit, concentrations of corticosterone in serum or plasma (commercially available) were determined in the species listed in the table below. Corticosterone was detected in the all the species tested.

Species Tested	Amounts detected ng/mL
Human serum	116
Human plasma	107
Mouse serum	48
Rat serum	92
Rabbit serum	199
Cyno serum	134
Bovine serum	45
Porcine serum	89
Horse serum	35
Chicken serum	109

Troubleshooting Guide

You will find detailed recommendations for common situations you might encounter with your AlphaLISA Assay kit at: www.revvity.com

RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES



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