CORTISOL SPARCL™ ASSAY
Life Diagnostics, Inc., Catalog Number: CORT-SP

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMAN OR VETERINARY DIAGNOSTICS

INTRODUCTION

Cortisol is a glucocorticoid steroid hormone. It is produced in the adrenal cortex and released into the blood in response to stress. It aids in regulation of glucose levels, immune response and cardiovascular function.

PRINCIPLE OF THE ASSAY

The cortisol SPARCL™[1] (Spatial Proximity Analyte Reagent Capture Luminescence, ref 1) assay uses a competitive format. Cortisol present in samples competes for binding of cortisol-HRP to a cortisol antibody that is conjugated to acridan, a chemiluminescent substrate. When cortisol-HRP binds to anti-cortisol-acridan, HRP and acridan are brought into close proximity. With the addition of hydrogen peroxide, HRP catalyzes oxidation of proximal acridan molecules causing a flash of chemiluminescence. Acridan conjugated antibody that is not bound to cortisol-HRP produces no signal. This principle allows the development of a no-wash assay that allows rapid measurement of cortisol concentrations.

During the assay, HRP-cortisol is first added to wells of a 96-well SPARCL™ plate[2]. Cortisol standards and diluted samples are then added. After brief mixing, anti-cortisol-acridan is added and the plate incubated on a shaker at 25°C and 150 rpm. After thirty minutes the plate is placed into a luminometer. Trigger solution containing hydrogen peroxide is injected into each well and luminescence is immediately measured. The concentration of cortisol is inversely proportional to luminescence and is derived from a standard curve.

MATERIALS AND COMPONENTS

Materials provided with the kit*:*

- Cortisol-HRP stock **Store ≤ -70°C**
- Anti-cortisol acridan stock **Store ≤ -70°C**
- Cortisol stock[3] **Store ≤ -70°C**
- 10 ml Dissociation buffer; SDB10-1, 10 ml
- Diluent; CSD50-1, 2 x 50 ml
- Trigger solution; TS7-1, 7 ml
- White SPARCL™ plate: 12 x 8-well
- Clear untreated 96-well plate

Materials required but not provided:

- Precision pipettes and tips
- Polypropylene tubes
- Vortex mixer
- Plate incubator/shaker
- Luminometer capable of simultaneous injection & measurement
- Curve fitting software

STORAGE

Store the cortisol-HRP, anti-cortisol-acridan and cortisol stock vials at or below -70°C (they may be stored at -20°C for one week). The remainder of the kit should be stored at 2-8°C. The SPARCL™ plate should be kept in a sealed bag with desiccant and antioxidant. The kit will remain stable for at least six months from the date of purchase, provided that the components are stored as described.

GENERAL INSTRUCTIONS

1. The dilution buffer, dissociation buffer and 8-well strips used in the assay should be allowed to reach room temperature (25°C) before use.
2. It is normal for crystals to form in the dissociation buffer. They can be dissolved by placing the sealed bottle in a beaker of lukewarm water and occasionally agitating the bottle.
3. It is important that reagents be added to the SPARCL™ plate in a timely manner. Cortisol-HRP and diluted samples should be added to the plate and mixed within 5 min. Acridan conjugate must be added to the plate within an additional 5 min prior to the 30-minute incubation.

STANDARD PREPARATION

Thaw the cortisol stock at room temperature.

Test tube method

1. Label 8 polypropylene tubes as 25, 16.67, 11.11, 7.41, 4.94, 3.29, 2.19 and 1.46 nM.
2. In the tube labeled 25 nM, prepare the 25 nM standard as described on the cortisol stock vial label.
3. Dispense 200 µl of diluent: CSD50-1, into the tubes labeled 16.67, 11.11, 7.41, 4.94, 3.29, 2.19 and 1.46 nM.
4. Pipette 400 µl of the 25 nM cortisol standard into the tube labeled 16.67 nM and mix. This provides the 16.67 nM cortisol standard.
5. Similarly prepare the remaining standards by 1.5-fold serial dilution.

96-Well plate method.

We recommend that standards and samples be prepared or aliquoted into a clear 96-well plate in the format planned for the SPARCL™ assay. This allows rapid transfer of standards and samples to the SPARCL™ plate using a multipipettor. Standards may be prepared in the clear 96-well plate as follows.

1. Prepare the 25 nM standard in a microcentrifuge tube as described on the cortisol stock vial label.
2. Dispense 100 µl of diluent CSD50-1 into wells B1-3 to H1-3
3. Pipette 300 µl of the 25 nM cortisol standard into wells A1-3.
4. Using a multipipettor, transfer 200 µl of the 25 nM cortisol standard from wells A1-3 to wells B1-3 and mix. This provides the 16.67 nM cortisol standard.
5. Similarly prepare the 11.11, 7.41, 4.94, 3.29, 2.19 and 1.46 nM standards by 1.5-fold serial dilution down the plate.

Use the standards within one hour of preparation.

SAMPLE PREPARATION

Serum and fluid samples. Prior to testing, serum and peritoneal fluid samples must be treated with dissociation buffer: SDB10-1 to dissociate cortisol from cortisol binding globulin. Use the following procedure for each sample.

[1] SPARCL technology was developed by Lumigen Corp.

[2] The plate provided with the kit has been treated with a reagent that reduces background chemiluminescence. Untreated plates cannot be used.

[3] Prepared from Cerilliant cortisol standard solution.

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Step 1. In a microcentrifuge tube mix 50.0 µl of sample with 50.0 µl of dissociation buffer: SDB10-1. Cap the tube and incubate for at least 10 minutes at room temperature. At this point, the sample has been diluted 2-fold.

Step 2. The dissociated samples must be further diluted at least an additional 20-fold in order to avoid matrix effects attributable to the dissociation buffer. This can be achieved by mixing 25 µl of the dissociated sample prepared in step 1, with 475 µl of CSD50-1 diluent. Please note that this represents a 40-fold dilution of the original sample. If further dilution is needed, use diluent CSD50-1.

Saliva and Urine. Pretreatment with dissociation buffer is not necessary for saliva and urine. However, when testing human samples, we found that saliva and urine had to be diluted at least 10-fold and 20-fold respectively, with CSD50-1, in order to eliminate matrix effects. Optimal dilutions must be determined by the end user. After preparation, we recommend that the diluted samples be aliquoted into appropriate wells of the clear 96-well polystyrene plate. This allows rapid transfer of 50 µl aliquots to the SPARCL™ plate using a multipipettor. If using this method, ensure that an excess volume is aliquoted into the clear plate in order to ensure complete transfer of 50 µl aliquots to the SPARCL™ plate.

CONJUGATE MIX PREPARATION

Instructions for preparation of the working HRP-cortisol conjugate stock and the anti-cortisol-acridan conjugate stock are detailed on the respective stock vial labels. If necessary, after thawing, briefly centrifuge the stock vials in order to ensure that the contents are at the bottom of the tubes. Prepare the working stocks shortly before use using diluent: CSD50-1.

LUMINOMETER SETUP

1. The luminometer must be capable of injection and simultaneous measurement of luminescence without any delay.
2. Prime the luminometer injection port with 1 ml of trigger solution.
3. Place the injection needle into the injection port as needed for BMG luminometers.
4. Program the luminometer to inject 37.5 µl of trigger solution per well and to measure from time zero for 1 second (50 x 0.02 second intervals).
5. Define the format of the assay using the luminometer software.
6. Because the white SPARCL™ plate is provided as a 12 x 8-well strips, allowing use of fewer than 96-wells, make sure that the luminometer is programmed to inject trigger solution only into the wells being used.
7. We use a BMG LUMIstar Omega set at a gain of 3600. Optimal gain should be determined by the end user.
8. There are several of manufacturers of luminometers that are equipped to run a SPARCL™ assay. Please contact Life Diagnostics or Lumigen (www.lumigen.com) to discuss your luminometer.

PROCEDURE

1. Before starting the assay ensure that the luminometer is primed with trigger solution and that the injection needle is positioned in the injection port.
2. Secure the desired number of SPARCL™ 8-well strips in the holder. Immediately seal unused strips in the resealable bag with desiccant and antioxidant. Store unused strips at 2-8°C.
3. Aliquot 25.0 µl of HRP-cortisol mix into each well.
4. Dispense 50.0 µl of standards and diluted samples into the wells. We recommend that standards and samples be tested in triplicate.
5. Briefly mix the sample and HRP-cortisol for 10 seconds on an orbital shaker at 150 rpm.
6. Aliquot 25.0 µl of anti-cortisol-acridan mix into each well.
7. Incubate on an orbital micro-plate shaker at 150 rpm and 25°C for 30 minutes.
8. After the 30-minute incubation, place the plate in the luminometer and measure luminescence after injection of 37.5µl trigger solution
9. Remove the plate from the luminometer and discard the used strips. Keep the plate frame if future use is intended.

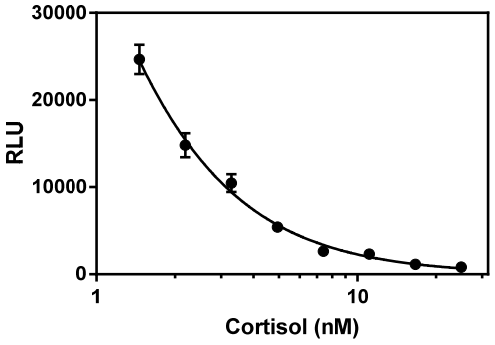
CALCULATION OF RESULTS

1. Before calculating results, review the raw data. If artefacts (RLU spikes) are apparent immediately after injection of trigger solution, eliminate that portion of the luminescence profile from analysis for all wells.
2. Using graphing software, construct a standard curve by plotting the luminescence (RLU) for the standards versus the $\log_{10}$ of the cortisol concentration.
3. Fit the data using a sigmoidal four-parameter logistic equation.
4. Derive the corresponding concentration of cortisol in the samples from the standard curve (remember to derive the concentration from the antilog).
5. Multiply the derived concentration by the dilution factor to determine the actual concentration of cortisol in the sample.
6. If the RLU values of diluted samples fall outside the standard curve, samples should be diluted appropriately and re-tested.
7. Values can be converted to ng/ml by multiplying nM values by 0.3625

TYPICAL STANDARD CURVE

A typical standard curve with RLU plotted on the Y-axis versus cortisol concentrations on the X-axis is shown below. This curve is for illustration only and should not be used to calculate unknowns.

Cortisol (nM)	RLU
25	808
16.67	1127
11.11	2314
7.41	2630
4.94	5412
3.29	10472
2.19	14809
1.46	24656



SPECIFICITY

The steroid hormones listed below were each tested over a concentration range of 1.95 – 250 nM in the CORT-SP assay and apparent concentrations calculated. The table lists the percent cross reactivity.

Steroid	% Cross reactivity
17-β-estradiol	0
Progesterone	6.7
Testosterone	0
11-Deoxycorticosterone	0
Prednisolone	65.3
Dexamethasone	0
Corticosterone	48.8

ACCURACY

Cortisol stripped serum was spiked with cortisol as indicated below. Cortisol concentrations were determined after dissociation and dilution.

Spiked concentration (nM)	Recovered concentration (nM)
800	822±76
400	426±52
200	215±25
100	117±25

REFERENCES

1. Akhavan-Tafti H. et al. A homogeneous chemiluminescent immunoassay method. J Am Chem Soc. 20;135(11):4191-4 (2013)

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For technical assistance please email us at
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