ab285353 – Human Cortisol (Saliva) Human ELISA Kit

For the quantitative measurement of Cortisol in saliva.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab285353

Storage and Stability

Kit can be used within one year if stored properly at -20°C. Avoid repeated freeze-thaw cycles. Return unused wells to the pouch containing desiccant pack, reseal along the entire edge.

Materials Supplied

Item	Quantity	Storage Condition
Plate Coated with Cortisol Ab	12 strips x 8 wells	-20°C
Assay Diluent	15 ml	-20°C
Wash Buffer (10x)	20 ml	-20°C
Standard Diluent	15 ml	-20°C
Cortisol Standard (4800 ng/ml)	500 µl	-20°C
Cortisol HRP Conjugate	1 vial	-20°C
TMB Substrate	11 ml	-20°C
Stop Solution	11 ml	-20°C
Plate Sealer	2	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 nm.
- Absorbent paper.
- Distilled or deionized water.

Reagent Preparation

- Briefly centrifuge small vials prior to opening.
- Read entire protocol before performing the assay.

Wash Buffer (10x): Dilute with deionized or distilled water to a final working 1x buffer concentration. If the Wash Buffer (10x) contains visible crystals, warm to room temperature and mix gently until dissolved before dilution.

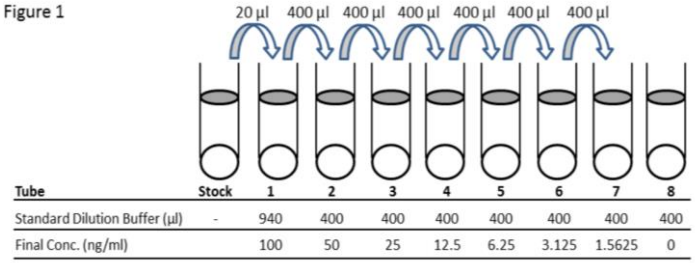
Cortisol HRP Conjugate: Reconstitute with 16 µl sterile dH2O to make 1000x stock solution. Let the vial sit at room temperature for 10 min. Tap gently to mix, make sure it is completely dissolved. Store the reconstituted Cortisol-HRP Conjugate solution at 4˚C. Use within two months

Standard Preparation

1. Prepare a series of dilutions for Cortisol Standard (4800 ng/ml) in Standard Diluent as shown in Figure 1.
2. Mix each tube gently and thoroughly before the next transfer.
3. Standard Diluent alone serves as the zero Standard (0 ng/ml).

Δ Note: *Discard unused diluted Standard solution*.

Figure 1



Tube	Stock	1	2	3	4	5	6	7	8
Standard Dilution Buffer (µl)	-	940	400	400	400	400	400	400	400
Final Conc. (ng/ml)		100	50	25	12.5	6.25	3.125	1.5625	0

Sample Preparation

1. Collect saliva sample and freeze/thaw at least once.
2. Centrifuge at 3000xg, room temperature for 5 min to remove the mucins (pellet) in sample.
3. Collect clear supernatant for the assay.

Assay Protocol

1. Bring all Buffers and desired number of Ab coated strips to room temperature (18-25°C) before use. It is recommended to run all Standard dilutions in duplicate.
2. Pipette 20 µl of Cortisol Standards or sample into their respective wells.
3. Make 1x solution of Cortisol HRP Conjugate (1000x) using Assay Diluent just before use. Make as much as needed. Add 100 µl of 1x Cortisol HRP Conjugate/well into appropriate wells. Cover wells with Plate Sealer and incubate with gentle shaking for 5 min. at room temperature. Incubate for 1 hr at 37°C.
4. Discard the solution. Wash 4 times (each time 3-4 min.) with 200 µl 1x Wash Buffer with gentle shaking.
5. Add 100 µl of TMB Substrate/well and gently shake. Measure absorbance at 650 nm for 5-30 min. at room temperature to monitor the blue color development, intensity of which is inversely proportional to the concentration of cortisol in the sample and Standards.

Δ Note: *Incubation time after addition of TMB substrate can be optimized to avoid over-development of color. Recommended absorbance for the 0 ng/ml cortisol standard is ~0.5-0.8 at 650 nm.*
Δ Note: *Optional: Prepare one parallel well for background control and add TMB Substrate.*

6. Add 100 µl of Stop Solution into each well including background control and mix with gentle shaking. Remove air bubbles if any. Measure absorbance at 450 nm within 5 min.

Calculation:

1. Calculate the mean absorbance for each set of duplicate Standards.
2. Plot Cortisol Standard Curve. Calculate Cortisol concentration of sample by interpolation of the Standard Curve.
3. If sample was diluted, multiply the value by dilution factor to calculate the concentration of Cortisol in the sample.

Δ Note: *Background subtraction for each reading is optional for calculating the sample Cortisol concentration and will not change the final results*.

Sample Cortisol Concentration = B x D = ng/ml where B is cortisol concentration in the sample well from Standard Curve. D is the sample dilution factor.

Technical Support

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