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4 Tiertz, N. W. , Text book of Clinical Chemistry, Saunders, 1968

2016-06-08

Cat#: CO116S-400 (96 tests)
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CALBIOTECH
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Sheep Cortisol ELISA

Catalog No. CO116S-400 (96 tests)

INTENDED USE

For Research Use Only. Not for use in diagnostic procedures.

MATERIALS PROVIDED	96 tests
1.Microwell coated with Cortisol MAb	12x8x1
2.Cortisol Standard: 7 vials (ready to use)	0.5 ml
3.Enzyme Conjugate (20X)	1.2 ml
4.Assay Diluent	24 ml
5.TMB Substrate: 1 bottle (ready to use)	12 ml
6.Stop Solution: 1 bottle (ready to use)	12 ml
7.20X Wash concentrate: 1 bottle	25 ml

MATERIALS NOT PROVIDED

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. Graph paper

STORAGE AND STABILITY

1. Store the kit at 2 – 8° C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.

WARNINGS AND PRECAUTIONS

1. For Research Use Only. Not for use in diagnostic procedures.
2. For laboratory use.
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
4. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
5. It is recommended that standards, control and serum samples be run in duplicate.
6. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

SPECIMEN COLLECTION HANDLING

1. Collect blood specimens and separate the serum immediately.
2. Typically, specimens may be stored refrigerated at (2-8° C) for 5 days. If storage time exceeds 5 days, store frozen at (-20° C) for up to one month.
3. Avoid multiple freeze-thaw cycles.
4. Prior to assay, frozen sera should be completely thawed and mixed well.
5. Do not use grossly lipemic specimens.

REAGENTS PREPARATION

1. Cortisol-enzyme Conjugate Solution

Dilute the Cortisol enzyme conjugate 1:21 with assay diluent in a suitable container. For example, dilute 100µl of conjugate with 2ml of assay diluent buffer for 10 wells (A slight excess of solution is made).

2. Wash Buffer

Prepare 1X Wash Buffer by adding the contents of the bottle (25ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (20-25°C).

ASSAY PROCEDURE

Prior to assay, allow reagents to stand at room temperature.
Gently mix all reagents before use.

1. Place the desired number of coated strips into the holder
2. Pipette 40 µl of Cortisol standards, control and patient's sera.
3. Add 200µl of Cortisol Enzyme Conjugate to all wells.
4. Incubate for 60 minutes at room temperature (20-25°C) with shaking.
5. Remove liquid from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbent paper towels.
6. Add 100 µl of TMB substrate to all wells.
7. Incubate for 15 minutes at room temperature (20-25°C) with shaking.
8. Add 50 µl of stop solution to all wells. Shake the plate gently to mix the solution.
9. Read absorbance on ELISA Reader at 450 nm within 20 minutes after adding the stop solution.

CALCULATION OF RESULTS

The standard curve is constructed as follows:

1. Check Cortisol standard value on each standard vial. This value might vary from lot to lot. Make sure you check the value on every kit. See example of the standard attached**.**
2. To construct the standard curve, plot the absorbance for Cortisol standards (vertical axis) versus Cortisol standard concentrations (horizontal axis) on a linear graph paper. Draw the best curve through the points.
3. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.

Example of a standard curve

Standard	Conc. (ng/ml)	OD (450 nm)
1	0	2.62
2	1	2.37
3	5	1.65
4	10	1.24
5	20	0.83
6	40	0.59
7	80	0.33

LIMITATIONS OF THE TEST

1. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.