LSBio
an Absolute Biotech Company

Goat Cortisol ELISA Kit (Competitive EIA)

User Manual

Catalog No. LS-F10174

It is important that you read this entire manual carefully before starting your experiment.

SAMPLE ONLY

This kit is for **Research Use Only**.
This kit is not approved for use in humans or for clinical diagnosis.

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ASSAY SPECIFICATIONS

Target:	Cortisol
Synonyms:	Cortisol
Specificity:	This kit is for the detection of Goat Cortisol. No significant cross-reactivity or interference between Cortisol and analogs was observed. This claim is limited by existing techniques therefore cross-reactivity may exist with untested analogs.
Sample Types:	This kit is intended for use with samples such as Plasma, Serum, and Tissue Homogenates. It has been empirically tested using the standard supplied with the kit (typically a recombinant protein).
Measurement:	Quantitative
Detection:	Colorimetric - 450nm
Detection Range:	3.12-200 ng/ml
Sensitivity:	Typically less than 1.56 ng/ml
Performance:	Intra-Assay CV=10%; Inter-Assay CV=20%
Limitations:	This kit is for **Research Use Only** and is not intended for diagnostic use. This kit is not approved for use in humans or for clinical diagnosis.

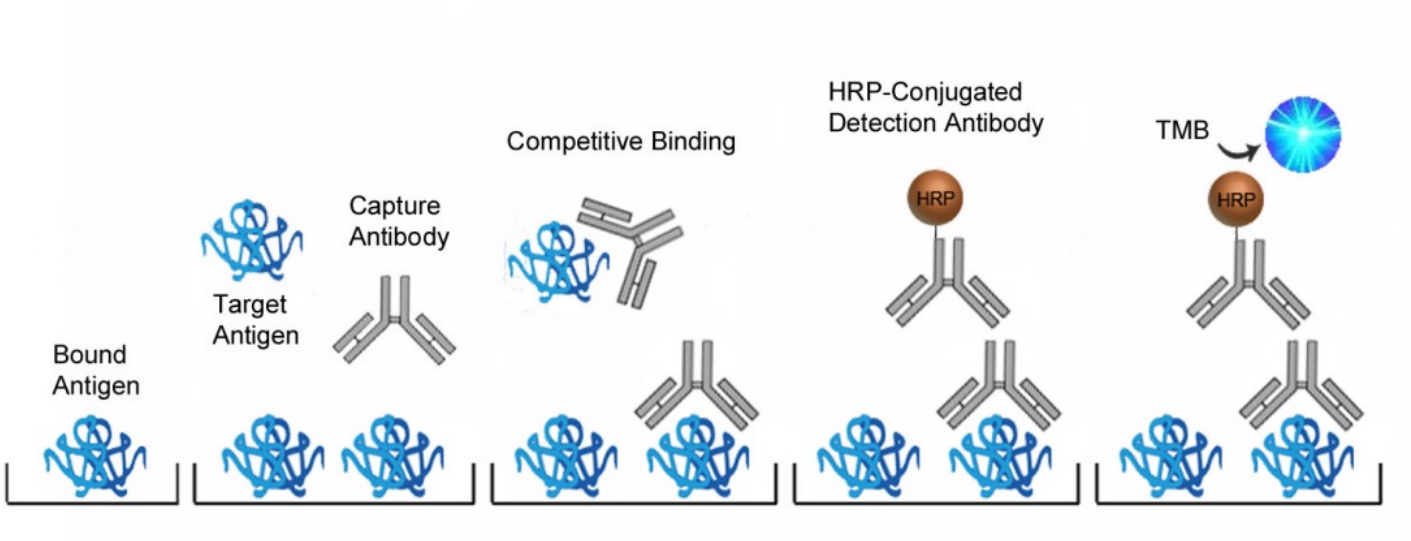
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Assay Principle

This assay is based on the competition EIA principle. Each well of the supplied microtiter plate has been pre-coated with target antigen. Standards or samples are added into the wells, along with a capture antibody that is specific for the target antigen. The antigen bound to the plate and the free antigen in the samples or standards compete for binding with this capture antibody. Antigen and antibody not bound to the plate are washed away. A Horseradish Peroxidase (HRP)-conjugated antibody is added that then binds to the capture antibody now bound to the plate. Unbound antibody is washed away and a TMB substrate is then added which reacts with the HRP enzyme resulting in color development. A sulfuric acid stop solution is added to terminate color development reaction and then the optical density (OD) of the well is measured at a wavelength of 450 nm ± 2 nm. The O.D. of an unknown sample can then be compared to an O.D. standard curve generated using known antigen concentrations in order to determine its antigen concentration. In contrast to typical Sandwich ELISA assays, in competition assays the greater the amount of antigen in the sample, the lower the color development and optical density reading.

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ASSAY PRINCIPLE IMAGE



KIT COMPONENTS AND STORAGE

Component	Quantity
Coated 96-well Assay Plate	1
Standards (Lyophilized)	2 vials
Capture Antibody (100x)	1 vial x 60 µl
Capture Antibody Diluent	1 vial x 10 ml
HRP-conjugate (100x)	1 vial x 120 µl
HRP-conjugate Diluent	1 vial x 20 ml
Sample Diluent	2 vial x 20 ml
Wash Buffer (25x)	1 vial x 20 ml
TMB Substrate	1 vial x 10 ml
Stop Solution	1 vial x 10 ml
Adhesive Plate Sealers	4
Instruction Manual	1

KIT STORAGE

The unopened kit can be stored at 2-8°C through the expiration date. Once opened, the kit can be stored at 2-8°C for 1 month. Unused strips should be kept in a sealed bag with the desiccant provided to minimize exposure to moisture.

OTHER REQUIRED SUPPLIES

- Microplate reader with 450nm wavelength filter with the correction wavelength set at 540nm or 570nm.
- High-precision pipette and sterile pipette tips
- Eppendorf tubes
- 37°C incubator
- Deionized or distilled water
- Absorbent paper

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EXPERIMENTAL LAYOUT

The following is an example of how to layout a study. A dilution series of the positive control Standard should be run in duplicate or triplicate, with the last well in each series being the negative control blank. Samples should also be run in duplicate or triplicate. Unknown samples should be run as a dilution series in order to identify the optimal dilution that produces an O.D. reading within the O.D. range of the positive control Standard dilution series.

Example 1: Standard Curve and dilution series of an unknown sample.

	1	2	3	4	...
A	Standard Dilution 1	Standard Dilution 1	Sample (1:1)	Sample (1:1)	...
B	Standard Dilution 2	Standard Dilution 2	Sample (1:10)	Sample (1:10)	...
C	Standard Dilution 3	Standard Dilution 3	Sample (1:100)	Sample (1:100)	...
D	Standard Dilution 4	Standard Dilution 4	Sample (1:1k)	Sample (1:1k)	...
E	Standard Dilution 5	Standard Dilution 5	Sample (1:10k)	Sample (1:10k)	...
F	Zero Standard	Zero Standard	Sample (1:100k)	Sample (1:100k)	...
G	Blank	Blank	Sample (1:1,000k)	Sample (1:1,000k)	...
H	Blank	Blank	Sample (1:10,000k)	Sample (1:10,000k)	...

Example 2: Standard Curve and samples run in duplicate.

	1	2	3	4	...
A	Standard Dilution 1	Standard Dilution 1	Sample A	Sample E	...
B	Standard Dilution 2	Standard Dilution 2	Sample A	Sample E	...
C	Standard Dilution 3	Standard Dilution 3	Sample B	Sample F	...
D	Standard Dilution 4	Standard Dilution 4	Sample B	Sample F	...
E	Standard Dilution 5	Standard Dilution 5	Sample C	Sample G	...
F	Zero Standard	Zero Standard	Sample C	Sample G	...
G	Blank	Blank	Sample D	Sample H	...
H	Blank	Blank	Sample D	Sample H	...

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REAGENT PREPARATION

Bring all reagents to room temperature (18-25°C) before use.

1x Capture Antibody: Centrifuge the vial before opening. Calculate the volume of 1x Capture Antibody solution needed for the number of wells to be run. Prepare this volume by diluting the supplied Capture Antibody Concentrate (100x) 100-fold with Capture Antibody Diluent.

1x HRP-conjugate: Centrifuge the vial before opening. Calculate the volume of 1x HRP-conjugate solution needed for the number of wells to be run. Prepare this volume by diluting the supplied HRP-conjugate Concentrate (100x) 100-fold with HRP-conjugate Diluent.

1x Wash Buffer: If crystals have formed in the concentrate, warm to room temperature and mix it gently until crystals have completely dissolved. Prepare 500 ml of 1x Wash Buffer by diluting the supplied 20 ml of 25x Wash Buffer Concentrate with 480 ml of deionized or distilled water. Wash Buffer can be stored at 4°C once prepared.

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REAGENT PREPARATION NOTES

1. All solutions prepared from concentrates are intended for one-time use. Do not reuse solutions.
2. Reagents may adhere to the tube wall or cap during transport so centrifuge tubes briefly before opening.
3. All solutions should be gently mixed prior to use.
4. To minimize imprecision caused by pipetting, ensure that pipettes are calibrated. Pipetting volumes of less than 10 µL is not recommended.
5. Substrate Solution is easily contaminated so sterility precautions should be taken. Substrate Solution should also be protected it from light.
6. Do not substitute reagents from one kit lot to another. Use only those reagents supplied within this kit.
7. Due to the antigen specificity of the antibodies used in this assay, native or recombinant proteins from other manufacturers may not be detected by this kit.

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SAMPLE COLLECTION AND STORAGE

This assay is recommended for use with goat tissue extracts.

Weigh out 2 grams of fresh clean green leaf tissue. Add 10ml Sample Extraction Buffer (1x) and grind using a mortar and pestle. Transfer the ground material to a microfuge tube, and incubate at 4°C for 30 minutes. Centrifuge for 10 minutes at 5,000 rpm. Draw off the supernatant (leaving the solids behind) and transfer to a clean tube for use in the assay.

SAMPLE COLLECTION NOTES

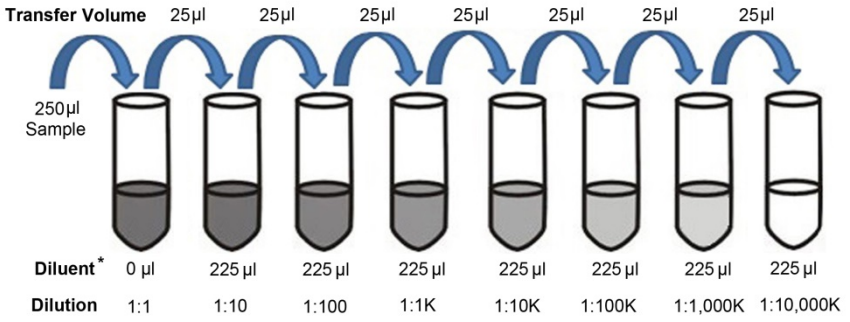
1. LSBio recommends that samples are used immediately upon preparation. Alternatively samples stored at 2-8°C should be used within 5 days. For long-term storage sample aliquots should be prepared and stored at -20°C if used within 1 month, or -80°C if used within 6 months. Long term storage can result in protein degradation and denaturalization, which may result in inaccurate results.
2. Avoid repeated freeze/thaw cycles for all samples.
3. Samples should be brought to room temperature (18-25°C) before performing the assay without the use of extra heating.
4. Sample concentrations should be predicted before being used in the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
5. LSBio is responsible for the quality and performance of the kit components but is NOT responsible for the performance of customer supplied samples used with the kit.

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SAMPLE PREPARATION

The resulting Optical Density (O.D.) values of your sample must fall within the O.D. values of the standard curve in order for the calculated antigen concentration to be accurate. In many cases samples will need to be diluted in order to lower the antigen concentration to sufficient levels. Information about antigen concentrations within various sample types may be available from the published literature; however, it is often necessary to run a dilution series of each sample type. The following will prepare sufficient volumes to run the Sample dilution series in triplicate. In the case of small volume samples the first step in the series can be a dilution, like 1:5 or 1:10, rather than undiluted sample. Running duplicate or triplicate wells for each sample is recommended.

* **Always dilute samples in the same buffer as the Standard used to generate the Standard Curve, in this case ddH_20**.



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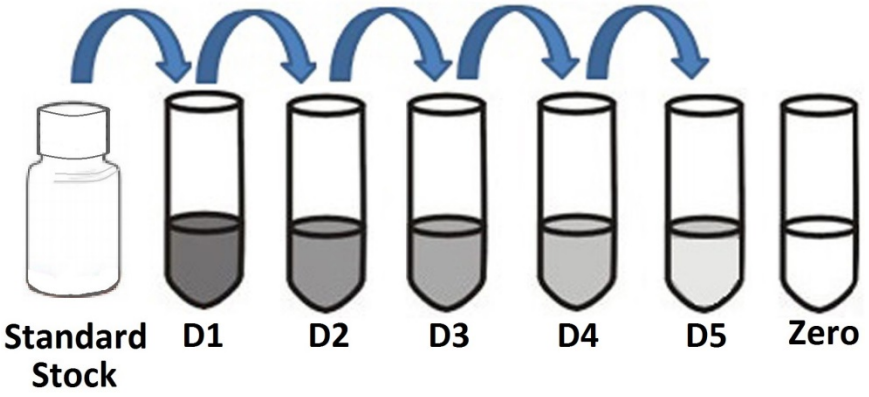
STANDARD PREPARATION

The following are instructions for the preparation of a Standard dilution series which will be used to generate the standard curve. The standard curve is then used to determine the concentration of target antigen in unknown samples (see the **Calculation of Results** section). The following will prepare sufficient volumes to run the Standard dilution series in duplicate. Reconstituted Standard and prepared standard dilutions should be used immediately and not stored for future use.

Standard Stock Solution (200 ng/ml): Reconstitute 1 tube of lyophilized Standard with 1.0 ml of Sample Diluent. Incubate at room temperature for 15 minutes with gentle agitation (avoid foaming).

D1 (200 ng/ml):	Pipette 300µl of Stock Standard into 0µl of Sample Diluent
D2 (100 µg/ml):	Pipette 150µl of D1 into 150µl of Sample Diluent
D3 (50 µg/ml):	Pipette 150µl of D2 into 150µl of Sample Diluent
D4 (25 µg/ml):	Pipette 150µl of D3 into 150µl of Sample Diluent
D5 (12.5 µg/ml):	Pipette 150µl of D4 into 150µl of Sample Diluent
D6 (6.25 µg/ml):	Pipette 150µl of D5 into 150µl of Sample Diluent
D7 (3.12 µg/ml):	Pipette 150µl of D6 into 150µl of Sample Diluent

Zero Standard (0 µg/ml): Use Sample Diluent alone



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ASSAY PROCEDURE

Bring all reagents and samples to room temperature without additional heating and mixed thoroughly by gently swirling before pipetting (avoid foaming). Prepare all reagents and samples as directed in the previous sections.

1. Set a Blank well without any solution.
2. Add 50 µl of **Standard** or **Sample** per well.
3. Add 50µl of **1x Capture Antibody** to each well (excluding the Blank well), gently agitate to ensure thorough mixing, cover with a plate sealer, and incubate for 40 minutes at 37°C.
4. Aspirate the liquid from each well and wash 3 times. Wash by adding approximately 200 µl of **1x Wash Buffer** using a squirt bottle, multi-channel pipette, manifold dispenser or automated washer. Allow each wash to sit for 2 minutes before completely aspirating. After the last wash, aspirating remove any remaining Wash Buffer then invert the plate and tap against clean absorbent paper.
5. Add 100 µl of **1x HRP-conjugate** to each well (excluding the Blank well), cover with a new plate sealer, and incubate for 30 minutes at 37°C.
6. Aspirate the liquid from each well and wash 5 times as outlined in step *4*.
7. Add 90µl of **TMB Substrate** to each well, gently agitate to ensure thorough mixing, and incubate **in the dark** for 20 minutes at 37°C.
8. Add 50 µl of **Stop Solution** to each well. The blue color will change to yellow immediately. If color change does not appear uniform, gently tap the plate to ensure thorough mixing. The Stop Solution should be added to wells in the same order and timing as was the substrate solution.
9. Determine the optical density (O.D. value) of each well immediately using a microplate reader set to 450nm with the correction wavelength set at 540nm or 570nm if available. Subtract the 540nm or 570nm reading from the 450nm reading to account for imperfections in the plate.

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ASSAY PROCEDURE NOTES

1. **ELISA Plate**: Keep appropriate numbers of strips for 1 experiment and remove extra strips from microtiter plate. Removed strips should be placed in a sealed bag containing desiccant, and stored at 4°C.
2. **Solutions**: To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
3. **Applying Solutions**: All solutions should be added to the bottom of the ELISA plate well. Avoid touching the inside wall of the well. Avoid foaming when possible.
4. **Assay Timing**: The interval between adding sample to the first and last wells should be minimized. Delays will increase the incubation time differential between wells, which will significantly affect the experiments accuracy and repeatability. For each step in the procedure, total dispensing time for addition of reagents or samples should not exceed 10 minutes.
5. **Incubation**: To prevent evaporation and ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods of time between incubation steps. Do not let wells dry out at any time during the assay. Strictly observe the recommended incubation times and temperatures.
6. **Washing**: Proper washing procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings. Residual liquid in the reaction wells should be patted dry against absorbent paper during the washing process. Do not put absorbent paper directly into the reaction wells.
7. **Controlling Substrate Reaction Time**: After the addition of the Substrate solutions, periodically monitor the color development. Stop color development before the color become too deep by adding Stop Solution. Excessively strong color will result in inaccurate absorbance reading.
8. **Reading**: The microplate reader should be preheated and programmed prior to use. Prior to taking O.D. readings, remove any

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residual liquid or fingerprints from the underside of the plate and confirm that there are no bubbles in the wells.

9. **Reaction Time Control**: Control reaction time should be strictly followed as outline.
10. **Stop Solution**: The Stop Solution is contains an acid, therefore proper precautions should be taken during its use, such as protection of the eyes, hands, face and clothing.
11. **Mixing**: During incubation times, the use of a micro-oscillator at low frequency is recommended. Sufficient and gentle mixing is particularly important in producing reliable result.
12. Kits from different batches may be a little different in detection range, sensitivity and color developing time. Please perform the experiment exactly according to the supplied instructions.
13. Due to inter- and intra-assay variability, it is recommended that appropriate carry-over controls be included between assays.
14. Prior to running valuable samples LSBio recommends that the user runs a preliminary experiment using the supplied controls in order to validate the assay.
15. To minimize extra influence on the performance, operation procedures and lab conditions, especially room temperature, air humidity, incubator temperature should be strictly controlled. It is also strongly suggested that the whole assay is performed by the same operator from the beginning to the end.
16. The kit should not be used beyond the expiration date on the kit label.

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ASSAY PROCEDURE SUMMARY

Prepare all reagents, samples and standards.

Set a **Blank** well with no solution.

Add 50 µl of **Standard** or **Sample** to each well.

Add 50 µl of **1x Capture Antibody** to each well (excluding the Blank) and incubate for 40 minutes at 37°C.

Aspirate and wash 3 times.

Add 100 µl of **1x HRP-conjugate** to each well (excluding the Blank) and incubate for 30 minutes at 37°C.

Aspirate and wash 5 times.

Add 90 µl of **TMB Substrate** to each well and incubate in the dark for 20 minutes at 37°C.

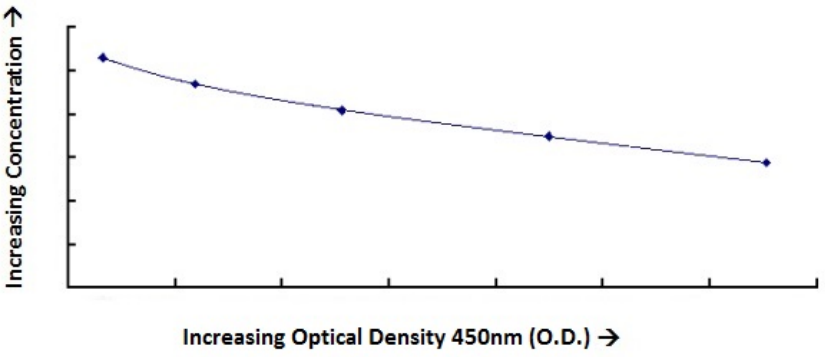
Add 50 µl **Stop Solution**.

Read immediately at 450nm.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample. Create a standard curve by plotting the mean absorbance for each standard on the X-axis against the target antigen concentration on the Y-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the target antigen concentration on the Y-axis versus the O.D. of the standards on the X-axis and the best fit line can be determined by regression analysis. The linear equation (Y = mx + b) can be used to calculate the standard curve where Y is the log of the concentration of the standard and x is the O.D. value of the standard. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Typical Data: The following standard curve is an example only and should not be used to calculate results for tested samples. A new standard curve must be generated for each set of samples tested.



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TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
Poor standard curve	Inaccurate pipetting.	Check pipettes.
	Wells not completely aspirated.	Completely aspirate wells between steps.
Low signal	Too brief incubation times.	Ensure sufficient incubation time.
	Incorrect assay temperature.	Use recommended incubation temperature. Bring substrate to room temperature before use.
	Inadequate reagent volumes.	Check pipettes and ensure correct preparation.
	Improper dilution.	
Deep color but low value	Plate reader settings not optimal.	Verify the wavelength and filter setting in the plate reader.
		Open the Plate Reader ahead to pre-heat.

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Troubleshooting Guide (continued)

Problem	Possible Cause	Solution
Large CV	Inaccurate pipetting.	Check pipettes.
High background	Plate is insufficiently washed.	Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed.
	Contaminated wash buffer.	Make fresh wash buffer.
Low sensitivity	Improper storage of the ELISA kit.	All the reagents should be stored according to the instructions.
	Stop solution not added.	Stop solution should be added to each well before measurement.

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Important Note: During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. We recommend briefly centrifuging the vial to dislodge any liquid in the container's cap prior to opening.

Warning: This reagent may contain sodium azide and sulfuric acid. The chemical, physical, and toxicological properties of these materials have not been thoroughly investigated. Standard Laboratory Practices should be followed. Avoid skin and eye contact, inhalation, and ingestion. Sodium azide forms hydrazoic acid under acidic conditions and may react with lead or copper plumbing to form highly explosive metal azides. On disposal, flush with large volumes of water to prevent accumulation.

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