Datasheet for ABIN5564636

# Cortisol ELISA Kit

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## Overview

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| Quantity: | 96 tests |
| Target: | Cortisol |
| Reactivity: | Chemical |
| Method Type: | Competition ELISA |
| Detection Range: | 0.195-200 ng/mL |
| Minimum Detection Limit: | 0.195 ng/mL |
| Application: | ELISA |

## Product Details

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| Purpose: | The AssayMax™ Cortisol ELISA (Enzyme-Linked Immunosorbent Assay) Kit is designed for detection of cortisol in plasma, serum, urine, milk, saliva, and cell culture samples. This assay employs a quantitative competitive enzyme immunoassay technique that measures cortisol in appoximately 3 hours. A polyclonal antibody specific for cortisol has been pre-coated onto a 96-well microplate with removable strips. Cortisol in standards and samples is competed with a biotinylated cortisol protein sandwiched by the immobilized antibody and streptavidin-peroxidase (SP) conjugate. All unbound material is washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured. |
| Brand: | AssayMax™ |
| Sample Type: | Cell Culture Cells, Milk, Plasma, Saliva, Serum, Urine |
| Analytical Method: | Quantitative |

## Product Details

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| Detection Method: | Colorimetric |
| Components: | Cortisol Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against cortisol. Sealing Tapes: Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay. Cortisol Standard: Cortisol in a buffered protein base (200 ng/ml, 0.5 ml). Biotinylated Cortisol Protein (3x): 1 vial, lyophilized. EIA Diluent Concentrate (10x): A 10-fold concentrated buffered protein base (20 ml). Wash Buffer Concentrate (20x): A 20-fold concentrated buffered surfactant (30 ml). SP Conjugate (100x): A 100-fold concentrate (80 l). Chromogen Substrate (1x): A stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml). Stop Solution (1x): A 0.5 N hydrochloric acid solution to stop the chromogen substrate reaction (12 ml). |
| Material not included: | Microplate reader capable of measuring absorbance at 405 nm. Pipettes (1-20 µL, 20-200 µL, and multiple channel). Deionized or distilled reagent grade water Incubator (37 °C) |

## Target Details

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| Target: | Cortisol |
| Abstract: | Cortisol Products |
| Target Type: | Hormone |
| Background: | Cortisol is a steroid hormone which belongs to the glucocorticoid class of hormones. When used as a medication, it is known as hydrocortisone. It is the main glucocorticoid secreted by the adrenal cortex, within the adrenal gland. Cortisol is released in response to stress and low blood-glucose concentration. Cortisol binds to the cytosolic glucocorticoid receptor and translocates into the cell nucleus, where it binds to many glucocorticoid response elements in the promoter region of the target genes. Cortisol promotes protein catabolism, gluconeogenesis, capillary wall stability, and renal excretion of calcium. It also suppresses immune and inflammatory responses. Cortisol regulates or supports a variety of important cardiovascular, metabolic, immunologic, and homeostatic functions (1-2). |

## Application Details

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| Assay Time: | 3 h |
| Plate: | Pre-coated |
| Protocol: | • Step 1. Add 25 µL of Standard or Sample and 25 µL of Biotinylated Protein per well. Incubate 2 hours. |

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| | • Step 2. Wash, then add 50 µL of SP Conjugate per well. Incubate 30 minutes.<br>• Step 3. Wash, then add 50 µL of Chromogen Substrate per well. Incubate 15 minutes.<br>• Step 4. Add 50 µL of Stop Solution per well. Read at 450 nm immediately. |
| Reagent Preparation: | Freshly dilute all reagents and bring all reagents to room temperature before use. EIA Diluent Concentrate (10x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the EIA Diluent Concentrate 10-fold with reagent grade water to produce a 1x solution. Store for up to 30 days at 2-8 °C. Cortisol Standard: Allow the vial to warm to room temperature prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting from the standard stock solution (200 ng/mL) 4-fold with EIA Diluent to produce 50, 12.5, 3.125, 0.781, and 0.195 ng/mL solutions. EIA Diluent serves as the zero standard (0 ng/mL). Any remaining stock solution should be stored at -20 °C. Avoid repeated freeze-thaw cycles. Standard Point Dilution [Cortisol] (ng/mL) P1 1 part Standard (200 ng/mL) 200 P2 1 part P1 + 3 parts EIA Diluent 50 P3 1 part P2 + 3 parts EIA Diluent 12.5 P4 1 part P3 + 3 parts EIA Diluent 3.125 P5 1 part P4 + 3 parts EIA Diluent 0.781 P6 1 part P5 + 3 parts EIA Diluent 0.195 P7 EIA Diluent 0.0 5 Biotinylated Cortisol Protein (3x): Reconstitute the Biotinylated Cortisol Protein with 5 mL of EIA Diluent to produce a stock solution. Allow the vial to sit for 10 minutes with gentle agitation prior to dilution. From the stock solution, dilute 3-fold with EIA Diluent to produce a 1x working solution. Any remaining stock solution should be stored at -20 °C and used within 30 days. Avoid repeated freeze-thaw cycles. Wash Buffer Concentrate (20x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 20-fold with reagent grade water to produce a 1x solution. SP Conjugate (100x): Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 100-fold with EIA Diluent to produce a 1x solution. The undiluted conjugate should be stored at -20 °C. |
| Sample Collection: | Plasma: Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x g for 10 minutes and collect plasma. A 20-fold sample dilution is suggested into EIA Diluent, however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20 °C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as an anticoagulant). Serum: Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x g for 10 minutes and remove serum. A 20-fold sample dilution is suggested into EIA Diluent, however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20 °C or below for up to 3 months. Avoid repeated freeze-thaw cycles. Urine: Collect urine using sample pot. Centrifuge samples at 800 x g for 10 minutes. A 2-fold sample dilution is suggested into EIA |

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| | Diluent, however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20 °C or below for up to 3 months. Avoid repeated freeze-thaw cycles. Saliva: Collect saliva using sample tube. Centrifuge samples at 800 x g for 10 minutes. An 8-fold sample dilution is suggested into EIA Diluent, however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20 °C or below for up to 3 months. Avoid repeated freeze-thaw cycles. Milk: Collect milk using sample tube. Centrifuge samples at 800 x g for 10 minutes. An 8-fold sample dilution is suggested into EIA Diluent, however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20 °C or below for up to 3 months. Avoid repeated freeze-thaw cycles. Cell Culture Supernatants: Centrifuge cell culture media at 3000 x g for 10 minutes at 4 °C to remove debris and collect supernatants. Samples can be stored at -20 °C or below. Avoid repeated freeze-thaw cycles. |
| Assay Procedure: | Prepare all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25 °C). Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator. Add 25 l of Cortisol Standard or sample to each well, and immediately add 25 l of Biotinylated Cortisol Protein to each well (on top of the standard or sample). Gently tap plate to ensure thorough mixing. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition. Wash five times with 200 l of Wash Buffer manually. Invert the plate each time and decant the contents, hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 l of Wash Buffer and then invert the plate, decanting the contents, hit 4-5 times on absorbent material to completely remove the liquid. Add 50 l of SP Conjugate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance. Wash the microplate as described above. Add 50 l of Chromogen Substrate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Incubate for 15 minutes or until the optimal blue color density develops. Add 50 l of Stop Solution to each well. The color will change from blue to yellow. Gently tap plate to ensure thorough mixing. Break any bubbles that may have formed. 6 Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at low concentration points after stopping the |

## Application Details

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| | reaction for about 10 minutes, which will reduce the readings. |
| Calculation of Results: | • Calculate the mean value of the duplicate or triplicate readings for each standard and sample.<br>• To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance (OD) on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit.<br>• Determine the unknown sample concentration from the standard curve and multiply the value by the dilution factor. |
| Restrictions: | For Research Use only |

## Handling

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| Handling Advice: | This product is for Research Use Only and is not intended for use in diagnostic procedures. Prepare all reagents (diluent buffer, wash buffer, standard, biotinylated protein, and SP conjugate) as instructed, prior to running the assay. Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this insert. However, the user should determine the optimal dilution factor. Spin down the standard and SP conjugate vials before opening and using contents. The Stop Solution is an acidic solution. Cortisol ELISA Kit Catalog No. EC3021-1 Sample insert for reference use only 2 The kit should not be used beyond the expiration date. |
| Storage: | 4 °C,-20 °C |
| Storage Comment: | Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date. Store Standard and SP Conjugate at -20°C. Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C. Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator. Diluent (1x) may be stored for up to 30 days at 2-8°C. Store Biotinylated Protein at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent. |

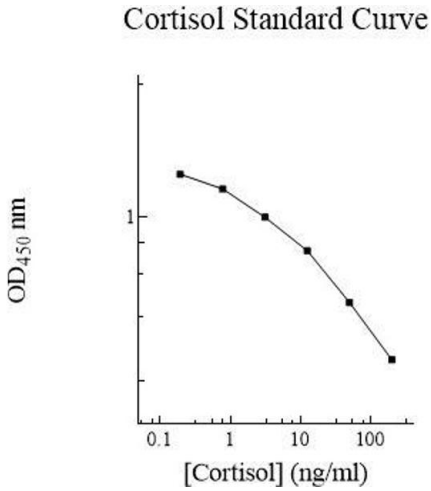


**Image 1.**