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User's Manual

Human Hydrocortisone ELISA kit

REF **DEIANJ18**

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

This kit is intended to be used for determination the level of HYD (hereafter termed "analyte") in undiluted original Human body fluids, tissue homogenates, secretions or feces samples.

General Description

Hydrocortisone, a corticosteroid, is similar to a natural hormone produced by your adrenal glands. It is often used to replace this chemical when your body does not make enough of it. It relieves inflammation (swelling, heat, redness, and pain) and is used to treat certain forms of arthritis; skin, blood, kidney, eye, thyroid, and intestinal disorders (e.g., colitis); severe allergies; and asthma. Hydrocortisone is also used to treat certain types of cancer.

Reagents And Materials Provided

Microelisa Stripplate 12×8 Strips

Standards×6 vials 0.5ml×6 vials

Sample Diluent 6.0ml

HRP-Conjugate Reagent 10.0ml

20× Wash Solution 25ml

Stop Solution 6.0ml

Chromogen Solution A 6.0ml

Chromogen Solution B 6.0ml

Closure Plate Membrane 2

Sealed Bags 1

Instruction 1

Materials Required But Not Supplied

Distilled or deionized water.

Absorbent papers or paper towels.

Pipettes and disposable pipette tips.

An ELISA reader capable of measuring absorbance at 450 nm.

A constant temperature incubator which can provide stable incubation conditions up to 37°C±0.5°C.

Storage

Store All Reagents At 2 - 8°C

Specimen Collection And Preparation

Serum - Centrifuge serum for approximately 20 minutes at 1000 ×g (or 3000 rpm) within 30 minutes after collection. Collect the supernatants carefully, assay immediately or store samples at -20°Cor -80°C. Avoid repeated freeze/thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for approximately 20 minutes at 1000 × g (or 3000 rpm) within 30 minutes after collection. Collect the supernatants carefully, assay immediately or store samples at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

Blood - Collect blood using EDTA or heparin as an anticoagulant. Centrifuge samples for approximately 15 minutes at 1500 ×g (or 5000 rpm) within 30 minutes after collection. Collect the supernatants carefully, assay immediately or store samples at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

Other Body Fluids (Lymph Fluid and Cerebrospinal Fluid) - Centrifuge samples for approximately 20 minutes at 1000 ×g (or 3000 rpm) within 30 minutes after collection. Collect the supernatants carefully, assay immediately or store samples at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

Tissue homogenates - The preparation of tissue homogenates will vary depending upon tissue type. Remove excess blood and weighed before homogenization. Minced the tissues to small pieces and homogenized them in a certain amount of PBS (Usually 10mg tissue to 100μl PBS.). After that, centrifugate homogenates for approximately 15 minutes at 1500×g (or 5000 rpm). Collect the supernatants carefully, assay immediately or store samples at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

Secretions (Saliva, Urine, Synovial Fluid and so on) - Centrifuge samples for approximately 20 minutes at 1000 ×g (or 3000 rpm) within 30 minutes after collection. Collect the supernatants carefully, assay immediately or store samples at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

Feces - Collect and fully shaking samples in a certain amount of PBS (Usually 10mg tissue to 100μl PBS.). After that, centrifugate homogenates for approximately 20 minutes at 1500×g (or 5000 rpm). Collect the supernatants carefully, assay immediately or store samples at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

Important Notes:

Although we have listed most of possible samples, but it does NOT mean the analyte exists in all of these listed samples, because some analytes only exist in some specific organelles, cells or tissues.

We are only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please make sure that sufficient samples are available.

Fresh samples without long time storage are recommended for assay. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results. Samples to be used within 5 days may be stored at 2-8°C, otherwise samples must be stored at -20°C(≤one month) or -80°C(≤two months) to avoid loss of bioactivity and contamination. Avoid repeated freeze/thaw cycles.

Grossly hemolyzed samples are not suitable for use in this assay, so the samples should be centrifugated adequately and no hemolysis or granule was allowed.

The kit can not assay the samples which contain sodium azide (NaN3), because NaN3 will inhibit the activity of horseradish peroxidase (HRP).

If the samples are not indicated in the manual, a preliminary experiment to determine the validity of this kit is necessary.

Reagent Preparation

Please store the plate and all reagents at 2°C-8°C.

1. The valid period of this kit is six months at 2°C-8°C. The kit should not be used beyond the expiration date.
2. Wash Solution (1×) - Dilute one volume of Wash Solution (20×) with nineteen volumes of deionized or distilled water. Diluted Wash Solution is stable for one month at 2°C-8°C. Undiluted Wash Solution and other reagents are stable for six months at 2°C-8°C.
3. When the kit is opened, please used up all Microelisa Stripplate as soon as possible after removed the plate from the foil pouch.

The Microelisa Stripplate is detachable, so please return the unused wells to the foil pouch containing the desiccant pack, and reseal

along entire edge of zip-seal for preventing damps. The remaining reagents still need to be stored at 2°C-8°C.

Assay Procedure

Please check all reagents and equipments before the experiments and make sure everything is right and OK!

Please do the experiments strictly to follow the assay procedures and DO NOT change any assay procedures arbitrarily!

1. Bring all reagents and samples to room temperature (18°C-25°C) naturally for 30min before starting assay procedures. DO NOT use hot water baths to thaw samples or reagents. If necessary, doing a low - speed centrifugation for one or two seconds to concentrate the Standards to the bottom of the vials. The Microelisa Stripplate is detachable, detach unused strips from the plate frame, return them to the foil pouch with the desiccant pack, and reseal for preventing damps.
2. Set Standard wells, Sample wells and Blank/Control wells, add Standard 50µl to each Standard well, add Sample 50µl to each Sample well, add Sample Diluent 50µl to each Blank/Control well. It is recommended that all Standards, samples and Sample Diluent be added in duplicate to the plate.
3. Add 100µl of HRP-conjugate reagent to each well, cover with a Closure Plate Membrane and incubate for 60 minutes at 37°C.
4. Wash the plate 4 times.
4. 1 Manual Washing - Dump the incubation mixtures of the wells into a sink or proper waste container. Using pipette or squirt bottle, fill each well completely with Wash Solution (1×), after about one minute's standing, invert and hit the plate onto absorbent papers or paper towels until no moisture appears. Repeat this procedure four times. Note: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame.
4. 2 Automated Washing - Aspirate all wells, then wash plates four times using Wash Buffer (1×). Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350µl/well/wash. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears.
5. Add Chromogen Solution A 50µl and Chromogen Solution B 50µl to each well successively. Then protect from light to incubate for 15 minutes at 37°C.
6. Add 50µl Stop Solution to each well. The color in the wells should change from blue to yellow.

7. Read the Optical Density (O.D.) at 450 nm using an ELISA reader within 15 minutes after adding Stop Solution (Around 5 minutes is the best time.).

Calculation

1. Average the duplicate readings for each standard and sample to subtract average optical density of the Blank/Control (VB/C).

Standards (concentration): 0 SI SII SIII SIV SV SVI

Mean O.D.(450nm): VB/C V1 V2 V3 V4 V5 V6

Adjusted: 0 V1-VB/C V2-VB/C V3-VB/C V4-VB/C V5-VB/C V6-VB/C

2. Using the professional curve fitting software to make a standard curve (usually most of the curves are linear, and a few curves are quadratic or cubic) and calculate the level of this analyte.
3. Note: Any variation in ambient temperature, equipment, operation, pipetting, washing, incubation temperature or time, and kit age can cause variation in result. Each user should obtain his own standard curve.

Specificity

No significant cross-reactivity or interference between this analyte and analogues is observed.

Precautions

1. Limited by current skill and knowledge, it is impossible for us to complete the cross-reactivity detection between this analyte and all it's analogues, therefore, cross reaction may still exist in other species or materials.
2. Influenced by the factors including cell viability, cell number and also sampling time, samples from cell culture supernatant are NOT suitable for detected by this kit.
3. The reagents and the plate of this kit and it's technical design parameters are only matched and designed for optimal performance for the undiluted original samples in this assay, and owing to the possibility of mismatching between antigen/antibody from other manufacturers and antibody/antigen that are used in this kit (such as difference in conformational epitope cased by chemical environment or difference in linear epitope, and so on), proteins extracted or recombinant proteins are NOT suitable for detected by this kit, and please do NOT substitute reagents from one kit to other kit and use only the reagents supplied by manufacturer, and moreover, we will NOT responsibility for using this kit or part of this kit to do any other experiments (such as western blot, immunohistochemistry, spike/recovery and so on) arbitrarily.
4. Each kit has been strictly passed Q.C test. However, results from end users might be inconsistent with our in house data due to some unexpected transportation or storage conditions, or different ambient temperature, lab equipment, operation, pipetting, washing, incubation temperature or time, and kit age. Assay variance among wells or kits might arise from these factors, too.
5. Kits from different manufacturers with the same item might produce different results, since different manufacturers can use different antigens or antibodies, and production processes.
6. The Stop Solution suggested for use of this kit is an acid solution, so please pay enough attention to safety when use it. Serum and plasma should be handled as potentially hazardous and capable of transmitting

disease. Disposable gloves must be worn during the assay procedure, since no known test method can offer complete assurance that products derived from blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.

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