BZERO Cortico

Enzyme immunoassay for the detection of corticosteroids (Cat.nr. HU0050008)

BZERO Cortico is a kit prepared for a competitive immunoenzymatic assay for the quantitative analysis of corticosteroids.
The kit contains the procedure and the materials sufficient for 96 determinations.
For result evaluation a microtiter plate or a strip photometer is required (manual or automatic ELISA reader).

Type of samples that can be analyzed (matrices)
Liver, muscle, milk, urine and feed.

Sample preparation
- Urine: centrifugation or filtration, dilution.
- Liver, muscle: homogenization, extraction, centrifugation, evaporation, reconstitution.
- Milk: centrifugation, extraction, centrifugation, evaporation, reconstitution.
- Feed: extraction, dilution.

Assay time: 75 minutes (sample preparation not included).

- **Detection limit**
- Urine: 0.25 ppb
- Liver, muscle: 0.2 ppb
- Milk: 0.05 ppb
- Feed: 5 ppb

Specificity	
Compound	**Cross-reactivity (%)**
Dexamethasone	100 %
Flumethasone	92 %
Betamethasone	70 %
Triamcinolone	21 %
Prednisolone	20 %
Cortisol	2.4 %
Cortisone	0.5 %
Metilprednisolone	0.2 %

1. TEST PRINCIPLE

The assay is performed in plastic microwells which have been coated with anti-rabbit antibodies. Corticosteroids standard, the samples, the enzyme conjugate and a specific anti-corticosteroids antibody are added to the microwells.
During the first incubation, free corticosteroids molecules and corticosteroid-HRP compete for the anti-corticosteroids antibodies binding sites. The anti-corticosteroids antibodies are simultaneously bound to the solid phase. Any unbound substance is then removed in a washing step.
The bound enzyme activity is determined by adding a fixed amount of a chromogenic substrate. The enzyme converts the colorless chromogen into a blue product during the second incubation.
The addition of the stop reagent leads to a color change from blue to yellow. The absorbance is measured by a microplate reader at 450_{nm}. The color development is inversely proportional to corticosteroids concentration in the standard/samples.

2. PROVIDED REAGENTS

Microtiter plate: 96 wells (12 strips x 8 wells, separable), coated with anti-rabbit IgG.
As the strips are breakable, the wells can be used individually. For this purpose, it is sufficient to get out the wells from the frame and to break the joint.
Std.0: 1 glass vial containing 3 ml of 0 ng/ml of dexamethasone standard solution.
ATTENTION: Only the zero standard is provided. B/B_0 values of calibration curve (0.01-0.5 ng/ml) are reported in the kit certificate of analysis.
Enzyme conjugate:1 plastic vial containing 200 µl, of concentrated enzyme conjugate.
Anti- corticosteroids antibody:1 plastic bottle containing 12 ml.
Dilution buffer 10x: 1 plastic bottle containing 50 ml.
Washing buffer 10X:1 plastic bottle containing 50 ml.
Developing solution:1 plastic bottle containing 24 ml.
Stop solution: 1 glass bottle containing 8 ml. White cap.

3. REQUIRED BUT NOT PROVIDED MATERIALS

- Distilled water
- Acetonitrile (liver, muscle, milk, feed)

Equipment
- Ultra-turrax (muscle, liver)

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- Low speed shaker (liver, muscle, milk, feed)
- Bench-centrifuge (urine; milk; liver and muscle)
- Alternatively filter paper type Whatman 1 (urine)
- Balance (muscle, feed, liver)
- Evaporation Apparatus (muscle, milk, liver).
- Micropipette 20-200 µl, tips.
- Micropipette 100-1000 µl, tips.
- Multichannel micropipette 50-250 µl,tips.
- Microplate reader, filter 450 nm.

4. WARNING AND PRECAUTIONS FOR THE USERS

- The test for *in vitro* diagnostic use only.
- Some reagents contain solutions that may be identified as dangerous substance by the Regulation (EC) No 1272/2008. Please refer to *Material Safety Data Sheet* available on both the Eurofins Technologies and Eurofins Tecna *(tecna.eurofins-technologies.com)* web site.
- Handle the reagents with caution, avoiding contact with skin, eyes and mucous membranes.

5. HANDLING AND STORAGE INSTRUCTIONS

- Store the kit at +2/+8 °C and never freeze.
- **Bring all reagents to room temperature before use (at least 1 hour). ATTENTION:** Do not unseal the microplate until it reaches the room temperature.
- Reseal the unused strips of the microtiter plate in the bag together with the desiccant bag provided.
- Return all reagents to +2/+8 °C immediately after use.
- Do not use components after the expiration date.
- Do not intermix components from different kit lots.
- Do not use photocopies of the instruction booklet. Follow the original instruction booklet that is included with the kit.
- Do not change the assay procedure, in particular:
  - do not prolong the incubation times;
  - do not incubate the plate at temperatures higher than 25°C;
  - do not shake the plate during the incubations.
- Use accurate and precise micropipettes with suitable tips for dispensing.
- Once started, complete all the steps without interruption.
- The reproducibility of ELISA results depends largely upon the efficiency and uniformity of microwells washing; always keep to the described procedure.
- Use a single disposable tip for each standard solution and sample to avoid cross-contamination.
- Do not allow tips to contact the liquid already in the microwells.
- Avoid exposure to direct light during all incubations. It is recommended to cover the microtiter plate without using plate sealers.

6. SAMPLES PREPARATION

6.1 Urine samples

1) Centrifuge the samples at 2000 g for 5 minutes or filtrate them (Whatman 1).
2) Dilute the supernatant 1:25 with dilution buffer 1x (i.g.: 100 µl supernatant + 2400 µl dilution buffer 1x).
3) The dilution factor is 25.

An alternative procedure with column purification is available upon request.

6.2 Liver and muscle samples

1) Homogenise an aliquot of sample with Ultraturrax or similar equipment.
2) Weigh 1 g of homogenised sample in a 10 ml test tube.
3) Add 5 ml of acetonitrile.
4) Mix for 30 minutes with an automatic equipment.
5) Centrifuge at 9500 g for 15 minutes at +2/+8 °C.
6) Transfer 2 ml of the supernatant in a glass tube and evaporate at 50°C under a slow air or nitrogen stream.
7) Dissolve the residue with 1 ml of dilution buffer 1x.
8) Take 50 µl of this solution and further dilute it with 350 µl of dilution buffer 1x.
9) The dilution factor is 20.

6.3 Milk samples

1) Centrifuge samples at 3000g at +2/+8 °C for 10 minutes.
2) Separate the fat (upper phase) from the skimmed milk (lower phase).
3) Dispense 1 ml of the skimmed milk in a 10 ml test tube.
4) Add 5 ml of acetonitrile.
5) Mix for 30 minutes with an automatic equipment.
6) Centrifuge at 9500 g for 15 minutes at +2/+8 °C.
7) Transfer 2 ml of the supernatant in a glass tube and evaporate at 50°C under a slow air or nitrogen stream.

8) Dissolve the residue with 2 ml of dilution buffer 1x.
9) The dilution factor is 5.

6.4 Feed samples

1) Add 10 ml of an acetonitrile: water mix (7:3; vol/vol) to 1 g of sample.
2) Shake for 15 minutes on a low speed shaker (400 rpm).
3) Let stay for 15 minutes.
4) Dilute the cleared supernatant 1:50 with dilution buffer 1x (i.g.: 20 µl + 980 µl of dilution buffer 1x).
5) The dilution factor is 500.

7. WORKING SOLUTIONS PREPARATION

Std 0: ready to use.
Dilution buffer 10x: Calculate and prepare the quantity required for enzyme conjugate and samples. Dilute the concentrated buffer 10x (1+9) with distilled water. **ATTENTION:** For dilution, withdraw the concentrated buffer by a sterile and disposable pipette; do not leave the bottle open.
ATTENTION: In presence of crystals, bring solution at room temperature in order to solve them completely
Enzyme conjugate: **ATTENTION:** In order to recover the total amount of the conjugate, before use, centrifuge the vial for some seconds at low speed *(spin-down).*
Calculate and prepare the quantity required for the analytical session. Dilute the conjugate **1/150** in the dilution buffer 1x (for example, 20 µl of enzyme conjugate concentrated + 2980 µl of dilution buffer 1x).
ATTENTION: In order not to take less than 20 µl of concentrate enzyme conjugate make two consecutive dilutions.
DO NOT VORTEX
Anti-Corticosteroids antibody: ready to use;
Washing buffer: dilute the concentrate 1:10 (1+9) with distilled water. **ATTENTION:** In presence of crystals, bring the solution at room temperature and stir in order to solve them completely.
The diluted washing buffer is stable at room temperature for 24 hours and at +2/+8°C for two weeks.
Developing solution: ready to use; this solution is light sensitive: keep away from direct light.
Stop solution: ready to use. **ATTENTION:** It contains 1M sulphuric acid. Handle with care; in case of contact flush immediately with plenty of water.

8. ASSAY PROCEDURE

1) Predispose an assay layout, recording the standard 0 and samples positions, taking into account that all have to be run in duplicate.
2) First incubation
   - Add 50 µl of each standard/ sample into the corresponding wells.
   - Using the multichannel pipet, add 50 µl of enzyme conjugate in each well.
   - Using the multichannel pipet, add 100 µl of antibody in each well.
   - Shake the plate gently with rotatory motion for few seconds.
   - Incubate 60 minutes at room temperature.
   - Do not prolong the first incubation time and do not use automatic shakers.
3) Washing
   - Pour the liquid out from the wells.
   - Fill completely all the wells with working wash solution using a squeeze bottle. Pour the liquid out from the wells.
   - Repeat the washing sequence four (4) times.
   - Remove the remaining droplets by tapping the microplate upside down vigorously against absorbent paper.

Do not allow the wells to dry out

4) Developing
   - Using the multichannel pipet, add 200 µl of developing solution to each well.
   - Mix thoroughly with rotatory motion for few seconds.
   - Incubate for 15 minutes at room temperature.
5) Using a multichannel pipet, add 50 µl of stop solution to each well and mix thoroughly with rotatory motion for few seconds.
6) Measure the absorbance at 450 nm.
7) Read within 60 minutes.

9. RESULTS CALCULATION

- Calculate the mean absorbance of standard 0 (B_0) and each sample.
- Divide the mean absorbance value of each sample by the mean absorbance of the standard 0 and multiply by 100; the standard 0 is thus made equal to 100% and all the other absorbance values are expressed as percentages:

$$\frac{\text{Sample absorbance}}{\text{Standard 0 } (B_0) \text{ absorbance}} \times 100 = \frac{B}{B_0}(\%)$$

- Enter the B/B_0 values provided for each standard (0.01; 0.03; 0.06; 0.125; 0.25; 0.5 ng/ml) in the kit certificate of analysis in a semi-logarithmic system of coordinates and draw the standard curve.
- Interpolate the B/B_0 value of each sample to the corresponding concentration from the calibration curve. Multiply this concentration for the dilution factor, as reported in chapter 6.

Please note: for results calculation it is suggest to use the "point to point" curve. Excel spreadsheet are available on Eurofins Tecna website tecna.eurofins-technologies.com and can be downloaded directly from the bottom of the product page.

10. EVALUATION OF RESULTS

After processing the results, it is necessary to verify the assay performance. The verification is performed by comparison of obtained data with those given in kit specifications (see chapter 11).
If the values are outside the specifications given, then the results of the test are not assured, therefore the corticosteroids concentration levels in the samples may not be valid.
In these cases it is advised to check the expiry date of the kit, the wavelength at which the reading was performed, as well as the procedure followed.
If operation errors are not identified as cause, contact our technical assistance.
In order to avoid false positive results it is necessary to adopt a decision limit (CCα), in specific for urine and liver. The decision limit varies according to the type of sample. It is suggested to determine a decision limit for each matrix routinely analyzed in your laboratory.

WARNING: Kit replacement will only be possible in case of return. The kit must be stored in its integral version at +2/+8°C.

11 KIT SPECIFICATIONS

11.1 Assay specification

Description	Specifications
B_0 absorbance	$\geq$ 0.7 OD_{450nm}

12. LIABILITY

Samples evaluated as positive using the kit have to be re-tested with a confirmation method.
Eurofins Technologies Hungary shall not be liable for any damages to the customer caused by the improper use of the kit and for any action undertaken as a consequence of results.
Eurofins Technologies Hungary shall not be liable for the unsafe use of the kit out of the current European safety regulations.