l'screen Cortico

Enzyme immunoassay for the detection of corticosteroids (Cat.nr. HU0050007)

l'screen **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,including standards.
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)
Urine, liver, muscle, milk 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 solutions or 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 samples/standards.

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.
Dexamethasone Std: 7 glass vials containing 1.5 ml of the following concentrations: 0 ng/ml; 0.01 ng/ml 0.03 ng/ml; 0.06 ng/ml; 0.125 ng/ml; 0.25 ng/ml; 0.5 ng/ml
Anti-corticosteroids antibody:1 plastic bottle containing 12 ml of antibody.
Enzyme conjugate:1 plastic vial containing 200 µl of concentrated enzyme conjugate.
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. MATERIALS REQUIRED BUT NOT PROVIDED

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

Equipment
- Ultra-turrax (muscle, liver)
- 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 is for *in vitro* diagnostic use only.
- Some reagents contain solutions that may be identified as dangerous substances by the Regulation (EC) *N° 1272/2008.*

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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 (e.g.: 20 µl + 980 µl of dilution buffer 1x).
5) The dilution factor is 500.

7. WORKING SOLUTIONS PREPARATION

Dexamethasone Std: ready to use;
ATTENTION: In presence of crystals, bring solutions at room temperature in order to solve them completely.
Dilution buffer 10x: Calculate and prepare the quantity required for enzyme conjugate and samples just before the beginning of the analysis. Dilute the concentrated buffer 1:10 (1+9) with distilled water. **ATTENTION**: For dilution, withdraw the concentrated buffer with a sterile and disposable pipette; do not leave the bottle open.
Enzyme conjugate: **ATTENTION:** In order to recover the total amount of the conjugate, before use, centrifuge the vial for a few seconds at low speed *(spin-down).*
Calculate and prepare the quantity required for the analytical session just before the beginning of it. Dilute the conjugate **1:150** with the dilution buffer 1x (e.g.: 20 µl of concentrated enzyme conjugate + 2980 µl of dilution buffer 1x).
ATTENTION: It is recommended to withdraw a volume of concentrated enzyme conjugate not minor than 20 µl.
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 it 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 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 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 the 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. CALCULATION OF RESULTS

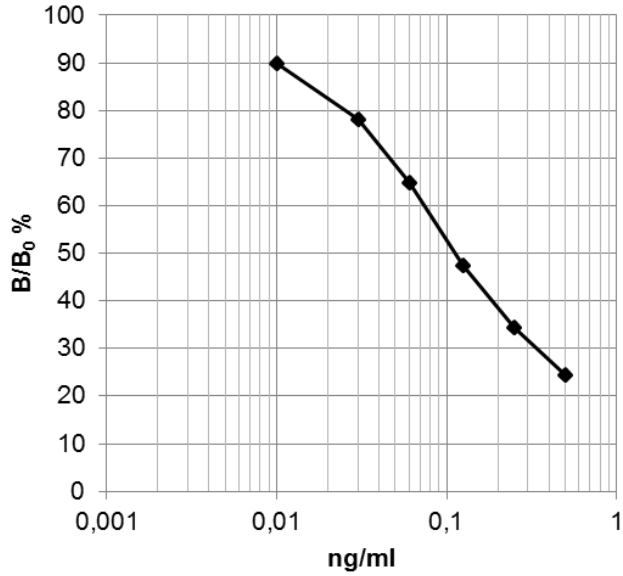
- Calculate the mean absorbance of each control, standard and sample.
- Divide the mean absorbance value of each standard and sample by the mean absorbance of the standard 0 (B_0) and multiply by 100; the standard 0 is thus made equal to 100% and all the other absorbance values are expressed as percentages:

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

- Enter the B/B_0 values calculated for each standard 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, Excel spreadsheets are available on Eurofins Tecna website tecna.eurofins-technologies.com and can be downloaded directly from the bottom of the product page.

10. CALIBRATION CURVE EXAMPLE



11. 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 12).
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 analysed in your laboratory.
In alternative, contact the technical assistance.

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

12.KIT SPECIFICATIONS

12.1 Assay specification

Description	Specifications
Mean B_0 absorbance	$\geq$ 0.7 OD_{450nm}
B/B_0 50 %	0.05 – 0.2 ng/ml
Std duplicates mean C.V.	$\leq$ 6 %

12.2 Assay performance

The kit performances hereby presented have been established in a collaborative validation study. Detection Capability (CCβ) was calculated as requested by of EU Decision 657/2002. A Performance Data Sheet (PDS) with more detailed information is available upon request.

Detection capability or CCβ (ppb)			
Matrix	**Dexamethasone**	**Betamethasone**	**Flumethasone**
Urine	2	2	2
Liver	1	1	1
Muscle	1	1	1
Milk	0.15	0.25	0.15

Matrix	Range (ppb)	Recoveries (%)
Liver	1-5	80 – 100
Liver	10-30	50 – 60
Feed	50	60 – 110

The results were obtained by means of a "4 parameters" elaboration of the calibration curve.

13.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.

14. LITERATURE

Falasca S.,. Gili M, Fioroni L., Stella C., Piersanti A., Galarini R.: "Validation of a new commercial ELISA screening test for corticosteroids detection in urine and liver". Fifth International Symposium on Hormone and Veterinary Drug Residue Analysis. Antwerpen, Belgium (May 16-19, 2006).

Cerni L., Biancotto G., Tondolo A. and Bogoni P.: " Dexamethasone and clenbuterol detection by enzyme immunoassay in bovine liver tissue: A new mutiresidue extraction procedure", Food and Agricultural Immunology (1998), vol 10, 307-315.