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I'screen CAP total

Enzyme immunoassay for the detection of chloramphenicol (code AB600)

***I'screen CAP total* (code AB600)** is a kit prepared for an immunoenzymatic assay for the quantitative analysis of CAP. The kit contains the procedures and materials sufficient for 96 determinations (standards included). A microtiter plate photometer or a strip photometer is required.

Analysable samples
Urine, serum, milk, muscle, seafood, honey, feed, eggs.

Sample preparation
Urine: centrifugation, dilution.
Milk: centrifugation.
Muscle, seafood (method I): homogenization, solvent extraction, centrifugation, evaporation.
Muscle, seafood (method II): homogenization, aqueous extraction, centrifugation.
Serum, honey: extraction, centrifugation, evaporation.
Feed: homogenization, solvent extraction, centrifugation, evaporation.
Eggs: homogenization, solvent extraction, centrifugation, evaporation.

Assay time: 60 minutes (sample preparation not included).

Detection limit
Urine: 0.6 ppb
Milk: 0.02 ng/ml
Muscle, seafood (method I): 0.04 ppb
Muscle, seafood (method II): 0.2 ppb
Honey: 0.04 ppb
Serum: 0.02 ppb
Feed: 0.04ppb
Eggs: 0.02 ppb

Specificity

Compound	Cross-reactivity (%)
Chloramphenicol (CAP)	100
CAP glucuronide*	85
Florfenicol	1
Tiamphenicol	1
CAP base	1

* Calculated in urine at B/B0 80%

1 TEST PRINCIPLE

The assay is performed in plastic microwells that have been coated with anti-CAP antibodies. CAP standard solutions or samples and the CAP-enzyme conjugate (HRP, horseradish peroxidase) are added to the microplate. During the first incubation, free CAP molecules and CAP-HRP compete for the binding sites of the anti-CAP antibodies bound to the solid phase. Any unbound enzyme conjugate is removed in a washing step. The bound enzyme activity is determined by adding a fixed amount of a chromogenic substrate. The enzyme converts the colourless chromogen into a blue product. The addition of the stop reagent leads to a colour change from blue to yellow. The absorbance is measured by a microplate reader at 450 nm. The colour development is inversely proportional to the CAP concentration in the standard or sample.

2 REAGENTS PROVIDED

Microtiter plate: 96 wells (12 strips x 8 wells), coated with anti-CAP antibodies.
As the strips are breakable, the wells can be used individually. For this purpose, it is sufficient to get out the wells from the sheath and to break the joint.
Chloramphenicol Std: 6 amber plastic vials, each containing 1.5 ml of standard solutions, in the following concentrations: 0 ng/ml; 0.02 ng/ml; 0.075 ng/ml; 0.25 ng/ml; 0.8 ng/ml; 2 ng/ml.
CAP spiking solution: 1 amber plastic vial containing 1 ml of 100 ng/ml of chloramphenicol.
Enzyme conjugate: 1 amber plastic vial containing 200 µl of the enzyme conjugate, 100x concentrated.
Enzyme conjugate diluent: 1 plastic bottle containing 12 ml, red solution.
Dilution buffer 5x: 1 plastic bottle containing 50 ml.
Washing buffer 10X: 1 plastic bottle containing 50 ml.
Developing solution: 1 amber plastic bottle containing 14 ml.
Stop solution: 1 glass bottle containing 8 ml. White cap.

3 MATERIALS REQUIRED BUT NOT PROVIDED

- Distilled water
- Ethylacetate (serum; honey; muscle and seafood method I; feed; eggs)
- Hexane (muscle and seafood method I; feed; eggs))
- Whatman n.1 filter paper

Equipment
- Centrifuge (better if refrigerated; milk, urine, serum, muscle, seafood, honey; feed; eggs)
- Vortex (muscle, seafood, honey, serum; feed; eggs)
- Homogenizer (muscle, seafood)
- Balance (muscle, seafood, honey; feed; eggs)
- Water bath (muscle and seafood method I; feed; eggs)
- Evaporator (serum; honey; muscle and seafood method I; feed; eggs)
- A 20-200 µl micropipette with tips.
- A multichannel 50 - 200 µl with tips.

- ELISA plate or strip reader equipped with a 450nm filter.

4 WARNING AND PRECAUTIONS FOR THE USERS

- For *in vitro* diagnostic use only.
- Some reagents contain solutions that may be identified as dangerous substance by the Regulation (EC) N° 1272/2008. Please refer to Safety Data Sheet available on Tecna 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 any component.
- Reseal the unused strips of the microtiter plate in the bag together with the desiccant bag provided.
- Do not use components after the expiration date.
- Do not intermix components between different kit lots.
- Do not use photocopies of the instruction booklet. Keep always to the instruction booklet included inside the kit.

6 SAMPLE PREPARATION

6.1 Urine

- Centrifuge the sample for 5 min at 2000g.
- Dilute the supernatant 30x with dilution buffer 1x (for example: 100 µl of sample + 2900 µl of dilution buffer)
- The dilution factor is 30.

ATTENTION: if an extraction procedure is preferred, please contact technical assistance at Support.ET.Trieste@eurofins.com for information about procedure.

6.2 Serum

- Add 2 ml of ethylacetate to 1 ml of serum and mix on vortex for 1 minute.
- Centrifugate 10 minute at 2000 g
- Transfer 1 ml of the upper layer (ethylacetate) in a glass tube and evaporate at 50°C under a nitrogen stream.
- Dissolve the residue is in 500 □l of dilution buffer 1X.
- The dilution factor is 1.

6.3 Milk

- Refrigerate the sample of raw milk and centrifuge it at +2/+8°C for 10 minutes at 3000g.
- Separate the fat from the skimmed milk.
- Test the skimmed milk directly in the assay.
- The dilution factor is 1.

ATTENTION: do not use preservatives containing sodium azide or chloramphenicol (ex. Azidiol) to stabilize milk samples. If a sample containing these stabilizers has to be analysed (for example, in case of proficiency tests), a solvent extraction procedure has to be applied. Contact technical assistance at Support.it.Trieste@eurofins.com

6.4 Muscle and seafood

Method I

- Homogenize the sample in a blender.
- Weigh 2 g of the homogenised sample and add 2 ml of distilled water. Mix well on vortex
- Add 4 ml of ethylacetate and shake vigorously for 10 minutes.
- Centrifuge at 2000g for 10 minutes
- Transfer 1 ml of the upper layer (ethylacetate) into a glass tube and evaporate to dryness (50°C) using a mild stream of nitrogen or air.
- Dissolve the residue in 1 ml of n-hexane and add 1 ml of dilution buffer 1x; mix on vortex for 1 minute.
- Centrifuge at 2000g for 10 minutes.
- In order to eliminate the emulsion in the interface, incubate the tube for 5 minutes in a water bath at 80°C, then centrifuge again in the same way.
- Transfer the aqueous lower phase in a new tube. The extract is ready for the assay.
- The dilution factor is 2.

Method II

- Homogenize the sample in a blender.
- Weigh 1 g of the homogenised sample and add 9 ml of dilution buffer 1x. Mix vigorously for 10 minutes
- Centrifuge at 2000g for 5 minutes
- Take the supernatant and filter it on a Whatman 1 filter paper. The filtrate is ready for the assay.
- The dilution factor is 10.

6.5 Honey

- Weigh 2 g of honey sample in a test tube and add 4 ml of distilled water. Mix on vortex until it is completely dissolved.
- Add 4 ml of ethyl acetate and mix vigorously for 10 minutes.
- Centrifuge for 10 min at 2000g
- Transfer 1 ml of ethyl acetate (it corresponds to the upper phase, which must be clear; if necessary, centrifuge again) in a glass tube and evaporate at 50 °C under a stream of nitrogen or air.
- Dissolve the residue on vortex with 1 ml of dilution buffer 1x. If any insoluble residues are present, centrifuge 1 minutes at 2000 g.
- The dilution factor is 2.

6.6 Feed

- Finely grind the sample.
- Weigh 1 g of sample.
- Add 4 ml of ethylacetate and shake vigorously for 5 minutes.
- Centrifuge at 2000g for 10 minutes
- Transfer 2 ml of the upper layer (ethylacetate) into a glass tube and evaporate to dryness (50°C) using a mild stream of nitrogen or air.
- Dissolve the residue in 1 ml of n-hexane and add 1 ml of distilled water. ATTENTION: if insoluble residues are present, do not discard them during transfer of the sample extract into centrifuge vials.
- Mix on vortex for 1 minute.
- Centrifuge at 2000g for 10 minutes.
- In order to eliminate the emulsion in the interface, incubate the tube for 5 minutes in a water bath at 80°C, then centrifuge again in the same way.
- Transfer the aqueous lower phase in a new tube. The extract is ready for the assay.
- The dilution factor is 2.
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6.7 Eggs

- Weigh 1 g of homogenised sample.
- Add 4 ml of ethylacetate and shake vigorously for 5 minutes.
- Centrifuge at 2000g for 10 minutes
- Transfer 2 ml of the upper layer (ethylacetate) into a glass tube and evaporate to dryness (50°C) using a mild stream of nitrogen or air.
- Dissolve the residue in 1 ml of n-hexane and add 0.5 ml of washing buffer 0.5x.
- Mix on vortex for 1 minute.
- Centrifuge at 2000g for 10 minutes.
- In order to eliminate the emulsion in the interface, incubate the tube for 5 minutes in a water bath at 80°C, then centrifuge again in the same way.
- Transfer the aqueous lower phase in a new tube. The extract is ready for the assay.
- The dilution factor is 1.

7 WORKING SOLUTIONS PREPARATION

Chloramphenicol Std: ready to use.
Enzyme conjugate diluent: ready to use.
Enzyme conjugate: *ATTENTION: in order to recover the total amount of the conjugate, centrifuge the vial for some seconds at low speed (spin-down) before use.*
Calculate and prepare the necessary quantity for the experiment, immediately before use, diluting the conjugate **1:100** in the enzyme diluent. DO NOT VORTEX.
Dilution buffer 5x: dilute the concentrate 1:5 (1+4) with distilled water.
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.
Stop solution: ready to use.

8 ASSAY PROCEDURE

8.1 Preliminary comments

- Bring all reagents at room temperature before use.
- Return all reagents at +2/+8 °C after use. Reseal the unused strips with the desiccant bag provided.
- Do not change the assay procedure, in particular:
  - do not prolong or shorten the first incubation time
  - do not incubate the assay at temperatures above 25°C or under 18°C
  - use always accurate and precise micropipettes with suitable tips
- Once started, complete all the steps without interruption.
- The reproducibility of ELISA results largely depends upon the efficiency and uniformity of microwells washing; always keep to the described procedure.
- To avoid cross-contamination, use a single disposable tip for each standard and sample.
- Do not allow tips to contact the liquid already present in the microwells or the inner microwells surface.
- Avoid direct sunlight during all incubations. Covering the microtiter plate is recommended.

8.2 Assay procedure

1. Predispose an assay layout, recording the Maximum Binding (standard 0 or B_O), standards and samples positions, taking into account that all have to be run in duplicate.
2. Add 50 µl of each standard/sample into the standard/sample wells.
3. Using a multichannel pipette, add 50 µl of the enzyme conjugate to all wells.
4. Incubate for 30 minutes at room temperature.

Do not prolong the first incubation time.

5. Washing sequence:
   - 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 4 times.
   - Remove the remaining droplets by tapping the microplate upside down vigorously against absorbent paper.

Do not allow the wells to dry out.

6. Using the multichannel micropipette, add 100 µl of developing solution to the wells and mix thoroughly with rotatory motion for few seconds.
7. Incubate for 30 minutes at room temperature, covering the plate from direct light.
8. Using a multichannel pipette, add 50 µl of stop solution to each well and shake the plate gently with rotatory motion for a few seconds.
9. Measure the absorbance at 450 nm. Read within 30 minutes.

In case an strip reader is used, it is necessary to take out the strip from the frame and to remove the case round the wells.

9 CALCULATION OF RESULTS

- Calculate the mean absorbance of standards and samples.
- Divide the mean absorbance value of each standard and sample by the mean absorbance of the Standard 0 (B_O) and multiply by 100; the Maximum Binding is thus made equal to 100% and the absorbance values are quoted in percentage:

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

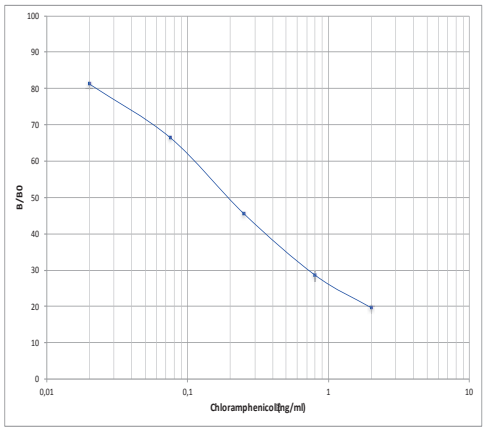
ATTENTION: for milk samples, multiply the calculated B/B0 values by a correction factor of 1.9.

- Enter the B/B_O values calculated for each standard in a semi-logarithmic system of coordinates and draw the standard curve.
- Interpolate B/B_O value for each sample to the corresponding concentration from the calibration curve.
- The concentration of chloramphenicol in the sample is to be calculated by multiplying the concentration read on the curve for the dilution factor according to sample preparation:
  - urine: 30
  - serum: 1
  - milk: 1

- muscle and seafood method I: 2
- muscle and seafood method II: 10
- honey: 2
- feed: 2
- eggs: 1

Please note: For results calculation, Excel spreadsheets are available on website and can be downloaded directly from the bottom of the product page.

10 EXAMPLE OF STANDARD CURVE



11 RESULTS EVALUATION

After results elaboration, it is necessary to verify the assay performance. The verification is performed by comparison of obtained data with those given in kit specifications (chapter 12). If the values are out from the specifications given, it is advised to check the expiry date of the kit, the wavelength of absorbance filter, as well as the procedure employed. If no errors are found, contact our technical assistance.
In order to avoid false positive results, it is necessary to adopt a decision limit (CCα). The value of this parameter is dependent upon matrix. For further information contact the technical assistance.
WARNING: substitution will be possible just in case of rendered kit. The kit must be conserved in its integral version and at the temperature indicated in this booklet.

12 KIT SPECIFICATIONS

12.1 Assay specifications

Mean Bo absorbance	$\geq$ 0.7 OD_{450nm}
B/Bo 50%	0.11-0.35 ng/ml
Std duplicates mean C.V.	$\leq$ 6 %

12.2 Assay performance

The kit performances hereby presented are from an in-house validation; Detection Capability (CCβ) was calculated as requested by of EU Decision 657/2002.

Detection Capability (CCβ)		
Matrix	**CAP**	**CAP-glucuronide**
Urine	1 ppb	1 ppb
Milk	0.1 ppb	
Muscle	Method I: 0.1 ppb	
Shrimp	Method I: 0.1 ppb	
Honey	0.15 ppb	
Feed	0.5 ppb	
Eggs	0.1 ppb	

Accuracy (recovery %)*		
Matrix	**CAP**	**CAP-glucuronide**
Urine	144±26	109±29
Milk	99±18	
Muscle	88±24	
Shrimp	109±26	
Honey	119±30	
Feed	108±31	
Eggs	71±34	

* spiked samples

13 LIABILITY

Samples evaluated as positive using the kit have to be re-tested with a confirmation method.
Tecna 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.
Tecna shall not be liable for the unsafe use of the kit out of the current European safety regulations.

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