Chloramphenicol ELISA Kit

Enzyme Immunoassay for the quantitative determination of Chloramphenicol in food and urine

Catalog number: ARG80792

Package: 96 wells

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INTRODUCTION

Due to its outstanding antibacterial properties, chloramphenicol is an often used antibiotic in the production of milk, meat and eggs. In humans it leads to haematotoxic side effects, like the chloramphenicol induced aplastic anemia. This has caused low limits in Germany, e.g. 1 µg/kg for milk, meat and eggs. Till now the chloramphenicol concentration was determined by radioimmunoassay or by gas chromatography. However, compared with conventional methods, enzyme immunoassays show some essential advantages. There is no need to work with radioactive material, the required assay time is shorter and the sensitivity is better than with chromatographic methods. The Chloramphenicol test provides a rapid, sensitive and reliable assay for the determination of chloramphenicol in food and urine. 40 samples can be assayed in duplicate within 60 minutes.

PRINCIPLE OF THE ASSAY

This is an Enzyme Immunoassay for the quantitative determination of Chloramphenicol in food and urine. This assay employs the sandwich quantitative enzyme immunoassay technique. An antibody specific for Chloramphenicol is bound onto a pre-coated microtiter plate. Chloramphenicol containing samples or standards are given into the wells of the microtiter plate. After incubation at room temperature, the wells are washed with diluted washing solution to remove unbound material. A peroxidase conjugated second antibody directed against Chloramphenicol is given into the wells and after incubation the plate is washed again. A substrate

solution is added and incubated for 15 minutes, resulting in the development of a blue color. The color development is inhibited by the addition of a stop solution, and the color turns yellow. The absorbance is proportional to the concentration of Chloramphenicol. A standard curve is constructed by plotting absorbance values against concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	12 strips x 8-well	4°C
Anti-Chloramphenicol antibody	6ml (ready to use)	4°C
Chloramphenicol Peroxidase conjugate	6ml (ready to use)	4°C
Standards	6 X 1 ml (ready to use) (0,0.05,0.1,0.5,1,5 ng/ml)	4°C
Sample dilution buffer	2X 60 ml	4°C
10x Wash Buffer	60 ml	4°C
TMB substrate	15 ml (ready to use)	4°C (Protect from light)
STOP solution	15 ml (ready to use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)
- Evaporator
- Ultra-Turrax, mixer
- Ethyl acetate
- N-Hexane
- Carrez I (Potassium hexacyanoferrate (II)-3-hydrate, 150g/L)
- Carrez II (Zincsulphate-7-hydrate,300g/L)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down all vials before use.
- If crystals are observed in the 10X Wash buffer, Extraction Buffer and Sample dilution buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Samples contain azide cannot be assayed.

SAMPLE COLLECTION & STORAGE INFORMATION

Milk (Direct assay): Refrigerate fresh milk samples at 2-8°C and centrifuge afterwards at 3000g for 10 mins. Remove upper fat layer and test the sample directly in the ELISA after warming to RT. For skimmed milk, the centrifugation step can be omitted. Samples dilution factor: F=1

Milk (Ethyl acetate extraction): Add 250µl Carrez I to 5ml milk sample, mix well and add 250µl Carrez II afterwards. Mix samples, refrigerate to 2-8°C and centrifuge at 3000g for 10 mins. Trasnfer 4.4ml of the clear supernatant to a clean vial, add 8ml ethyl acetate and agitate vigorously for 10 mins. For phase separation, centrifuge for 10 mins at 3000g at RT. Transfer 4ml of the upper ethyl acetate phase to a clean vial and evaporate the solvent at 50-70°C under a nitrogen stream to dryness. Dissolve the dry residue with 400µl sample diluent by shaking vigorously and test the sample in the ELISA. Sample dilution factor: F=0.2

Honey: Dissolve 2g honey in 4ml double distilled water. Add 4ml ethyl acetate and agitate vigorously for 10 mins. Transfer 1ml of the upper ethyl acetate phase to a clean glass vial and evaporate the solvent at 50-70°C under a nitrogen stream to dryness. Dissolve the dry residue with 500µl sample diluent by shaking vigorously and test the sample in the ELISA. Sample dilution factor: F=1

Shrimps, meat, fish meal: Mill and homogenize sample with an appropriate device. Mix 3g sample with 3ml double distilled water, add 6ml ethyl acetate and agitate vigorously for 10 mins. For phase separation, centrifuge for 10 mins at 3000g at RT. Transfer 4ml of the upper ethyl acetate phase to a clean vial and evaporate the solvent at 50-70°C under a nitrogen stream to dryness. Add 1ml n-Hexane to the residue. Add 500µl sample diluent to the mixture and agitate vigorously for 1 min. For phase separation, centrifuge for 10 mins at 3000g at RT. Test the lower, aqueous phase in the ELISA. Sample dilution factor: F=0.25

Whole egg (raw): Homogenize samples with an appropriate device (mixer, ultra-turrax). To 2g sample, add 12ml ethyl acetate and agitate vigorously for 10 mins. For phase separation, centrifuge for 10 mins at 3000g at RT. Transfer 6ml of the upper ethyl acetate phase to a clean vial and evaporate the solvent at 50-70°C under a nitrogen stream to dryness. Add 1ml n-Hexane to the residue. Add 1ml sample diluent to the mixture and agitate vigorously for 1 min. For phase separation, centrifuge for 10 mins at 3000g at RT. Test the lower, aqueous phase in the ELISA. Sample dilution factor: F=1

Urine: Centrifuge the samples at 3000g for 10 mins. Test the clear supernatant directly in the assay. Sample dilution factor: F=1

REAGENT PREPARATION

- 1X Wash buffer: Dilute 10X wash buffer into distilled water to yield 1X wash buffer.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 100 µl of standards and samples in duplicate into wells.
3. Add 50 µl anti-Chloramphenicol antibody into each well.
4. Incubate for 30 minutes at RT.
5. Without preceding washing, add 50 µl Chloramphenicol Peroxidase conjugate into each well.
6. Cover and incubate for additional 15 minutes at RT.
7. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with 1X wash buffer (350 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
8. Add 100 µl of TMB mixture to each well. Incubate for 15 minutes at room temperature in dark.
9. Add 100 µl of Stop Solution to each well.
10. Read the OD with a microplate reader at 450 nm immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

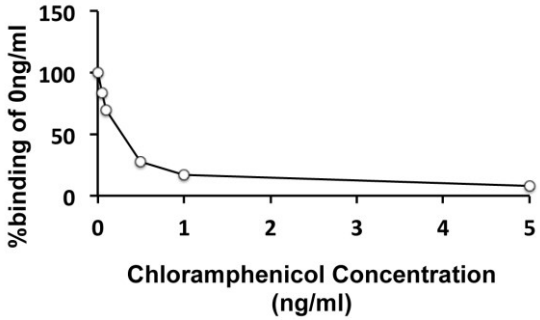
NOTE: Due to the extraction with ethyl acetate, negative samples may show a certain blank value. In repetitive performed experiments with negative samples for each matrix the following blank values were identified.

Milk (Direct assay)	< 0.1 ng/ml
Milk (ethyl acetate extraction)	< 0.1 ng/ml
Honey	< 0.2 ng/ml
Shrimps	< 0.2 ng/ml
Meat	< 0.2 ng/ml
Fish meal	< 0.2 ng/ml
Whole egg	< 0.05 ng/ml
Urine	< 0.2 ng/ml

These values are defined as the cut-off of the method for the respective matrices. Lower concentrations have to be considered as negative.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay. The binding is calculated as percentage of the absorption of 0ng/ml standard.



QUALITY ASSURANCE

Sensitivity

The sensitivity of the Chloramphenicol test is 0.03ng/ml with an assay range of 0.05-5 ng/ml.

Intra-assay precision

The CV value of intra-assay precision was 8%

Intra-assay precision

No cross-reactivity has been found with the following factors:

Chloramphenicol Base, Ampicillin, Penicillin, Tetracycline

Recovery

Mean recovery was determined by spiking samples with different amounts of Chloramphenicol:

Milk (direct assay)	94%
Milk (ethyl acetate extraction)	98%
Honey	98%
Shrimps	96%
Meat	108%
Fish meal	90%
Whole egg	95%
Urine	90%