Product information

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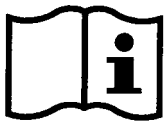

Useŕs Manual

Quinolones ELISA

Enzyme immunoassay for the rapid quantitative determination of Quinolones in food

REF **DEQUIE01**

Σ **96 wells**

RUO **For Research Use Only – Not for Use in Diagnostic Procedures**

Demeditec Diagnostics GmbH • Lise-Meitner-Straße 2 • 24145 Kiel (Germany)
Phone: +49 (0)431/71922-0 • Fax. +49 (0)431/71922-55
info@demeditec.de • www.demeditec.com

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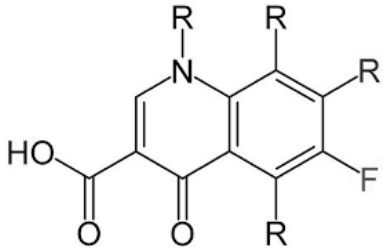
DMC
Updated 210119

Sensitivity	0.13 ng/mL
Recovery (spiked samples)	88 - 98%
Incubation Time	45 min

1. GENERAL INFORMATION



The **Quinolones ELISA** represents a highly sensitive detection system and is particularly capable of the rapid quantification of quinolone contaminations in milk, serum, egg, honey, shrimps, fish, meat and liver.

This test is designed for detection of substances within food products. This is not a medical device and is not intended to diagnose or prevent any diseases or other conditions.

2. PRINCIPLE OF THE TEST

The **Quinolones** quantitative test is based on the principle of the enzyme-linked immunosorbent assay. An antibody directed against quinolones is coated on the surface of a microtiter plate. Quinolones containing samples or standards and a quinolone-peroxidase conjugate are given into the wells of the microtiter plate. The conjugate competes with the quinolones of the samples/standards for the limited number of antibody sites. After 30 minutes incubation at room temperature the wells are washed with diluted washing solution to remove unbound material. A substrate solution is added and incubated for 15 minutes, resulting in the development of a blue colour. The colour development is inhibited by the addition of a stop solution, and the colour turns yellow. The yellow colour is measured photometrically at 450 nm. The concentration of quinolones is indirectly proportional to the colour intensity of the test sample.

3. PRECAUTIONS

Full compliance of the following good laboratory practices (GLP) will determine the reliability of the results:

1. Prior to beginning the assay procedure, bring all reagents to room temperature (20-25°C).
2. All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
3. Once the assay has been started, all subsequent steps should be completed without interruption and within the recommended time limits.
4. Replace caps in all the reagents immediately after use. Do not interchange vial stoppers.
5. Use a separate disposable tip for each specimen to prevent cross-contamination.
6. All specimens and standards should be run at the same time, so that all conditions of testing are the same.
7. Do not mix components from different batches.
8. Do not use reagents after expiration date.
9. Check both precision and accuracy of the laboratory equipment used during the procedure (micropipettes, ELISA reader etc.).

4. HEALTH AND SAFETY INSTRUCTIONS

1. Do not smoke or eat or drink or pipet by mouth in the laboratory.
2. Avoid contact of substrate and stop solution with skin and mucosa (possible irritation, burn or toxicity hazard). In case of contact, rinse the affected zone with plenty of water.
3. Handling and disposal of chemical products must be done according to good laboratory practices (GLP).

5. REAGENTS

The kit contains reagents for 96 determinations. They have to be stored at 2-8°C. Expiry data are found on the labels of the bottles and the outer package.

1. **SORB MT** Microtiter plate consisting of 12 strips with 8 breakable wells each, coated with anti-quinolone antibody.
2. **CAL 1 – 6** Ciprofloxacin Standards (0; 0.4; 1; 4; 10; 40 ng/mL): 6 vials with 1 mL each, dyed red, ready-to-use.
3. **ENZ CONJ** Conjugate (Quinolone-Peroxidase): 15 mL, dyed red, ready-to-use.
4. **SUB TMB** Substrate Solution (TMB): 15 mL, ready-to-use.
5. **STOP SOLN** Stop Solution (0.5 M H_2SO_4): 15 mL, ready-to-use.
6. **SAM DIL 5x** Sample Buffer (PBS): 120 mL as 5x concentrate, dyed red. Dilute 1+4 with distilled water. Stored at 4°C the diluted buffer is stable for at least one week. If during the cold storage crystals precipitate, the concentrate should be warmed up to 37°C for 15 minutes.
7. **WASH SOLN 10x** Washing Solution (PBS + Tween 20): 60 mL as 10x concentrate. Dilute 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up to 37°C for 15 minutes.
8. Instruction Manual.

6. ADDITIONAL INSTRUMENTATION AND REAGENTS (NOT PROVIDED)

Instrumentation

- 50 - 1000 µL micropipets
- Volumetric flask
- Analytical balance
- Mortar, mixer
- Centrifuge
- ELISA reader (450 nm)
- Water bath
- Plastic bag to store unused microtiter strips.

Reagents

- Double distilled water
- Methanol
- quinolones extraction buffer:
  Dilute 70% methanol (v/v) 1:2 (1+1) with **pre-diluted** sample buffer

7. SAMPLE PREPARATION

Honey

- 1 g of honey is suspended in 5 mL of 70% methanol (v/v).
- Mix suspension for 5 minutes at room temperature.
- Filter through Whatman #1 filter or alternatively centrifuge at a minimum of 3000 g for 15 minutes.
- Dilute 500 µL of filtrate/supernatant with 500 µL of **pre-diluted** sample buffer and test the sample in the ELISA.
  Dilution factor = 10

Milk / Serum / Other liquid samples

- Defat milk if applicable. Therefore the milk has to be centrifuged for 15 min at 4°C and at least 2000 g. Afterwards the upper fat layer should be removed.
- Dilute 1 mL of previously defatted milk or any other liquid sample in 9 mL of quinolones extraction buffer.
- Mix suspension for 5 minutes at room temperature.
- Filter through Whatman #1 filter or alternatively centrifuge at a minimum of 3000 g for 15 minutes. For a better separation of fat the centrifuge should be cooled to 4°C if applicable.
- Apply the filtrate / supernatant in the ELISA.
  Dilution factor = 10

All other solid samples

- To maximize homogeneity and representativeness of the sample drawing, a minimum of 5 g sample should be pulverized finely in a mortar, impact mill etc.
- 1 g of the homogenized mixture is suspended in 10 mL of quinolones extraction buffer.
- Mix suspension for 5 minutes at room temperature.
- Filter through Whatman #1 filter or alternatively centrifuge at a minimum of 3000 g for 15 minutes. For a better separation of fat the centrifuge should be cooled to 4°C if applicable.
- Apply the filtrate / supernatant in the ELISA.
  Dilution factor = 10

In case of too highly concentrated samples the sample extracts have to be further diluted with quinolones extraction buffer.

8. PROCEDURE

1. Prepare samples as described above.
2. Pipet 50 µL standards or prepared samples in duplicate into the appropriate wells of the microtiter plate.
3. Add 100 µL of quinolone-peroxidase conjugate into each well.
4. Incubate for 30 minutes at room temperature.
5. Wash the plate three times as follows: Discard the contents of the wells (dump or aspirate). Pipet 300 µL of diluted washing solution into each well. After the third repetition empty the wells again and remove residual liquid by striking the plate against a paper towel. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbances.
6. Pipet 100 µL of substrate solution into each well.
7. Allow the reaction to develop in the dark (e.g. cupboard or drawer; the chromogen is light-sensitive) for 15 minutes at room temperature.
8. Stop enzyme reaction by adding 100 µL of stop solution (0.5 M H_2SO_4) into each well. The blue colour will turn yellow upon addition.
9. After thorough mixing, measure absorbance at 450 nm (reference wavelength 620 nm), using an ELISA reader. The colour is stable for 30 minutes.

9. CALCULATION OF RESULTS

1. Calculate the average optical density (OD 450 nm) for each set of reference standards or samples.
2. Construct a standard curve by plotting the mean optical density obtained for each reference standard against its concentration in ng/mL on semi-log graph paper with the optical density on the vertical (y) axis and the concentration on the horizontal (x) axis. Alternatively the evaluation can be carried out by software. In this case the 4-parameter method should be preferred.
3. The diluted samples must be further converted by the appropriate **sample dilution factor** for calculating the sample concentration in ppb. The factors for each sample matrix are listed in the sample preparation section.

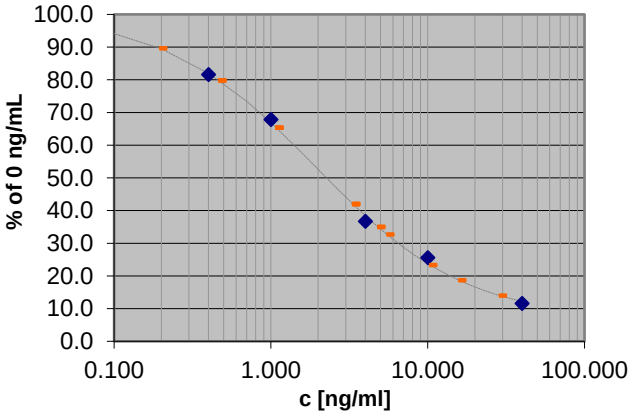
Example:
A honey sample prepared as described above results in 1.2 ng/mL. The concentration of the sample is calculated as follows:
C_{sample} = **1.2** (ng/mL) * **10** (ppb*ml/ng) = **12 ppb**

Additional dilution due to high sample concentration has to be accounted for.

10. TYPICAL STANDARD VALUES

The following table contains an example for a typical standard curve. The binding is calculated as percent of the absorption of the 0 ng/mL standard. These values are only an example and should not be used instead of the standard curve which has to be measured in each new test.

Ciprofloxacin (ng/mL)	% binding of 0 ng/mL
0	100
0.4	81
1.0	67
4.0	37
10	25
40	11



11. PERFORMANCE

Sensitivity
The limit of detection (LOD) of the **Quinolones test** is 0.13 ng/mL for the standard curve.
The limit of quantification (LOQ) of the **Quinolones test** is 0.42 ng/mL for the standard curve.

Validation experiments with common matrices resulted in the following LODs and LOQs [ppb].

Matrix	LOD	LOQ
Honey	1.6	4.0
Milk	1.5	5.6
Serum	1.2	4.1
Egg	2.2	5.0
Meat	1.5	5.1
Liver	2.2	4.3
Shrimps	2.8	5.8
Fish	1.2	5.0

Recovery

Honey	93%
Milk	96%
Serum	88%
Egg	92%
Meat	94%
Liver	98%
Shrimps	93%
Fish	92%

Linearity
The serial dilution of spiked matrices (as seen in the table Recovery) resulted in a dilution linearity of 71-92%.

Precision

Intra-assay Precision	4.3%
Inter-assay Precision	11.2%

Reactivity

Clinafloxacin	159%
Gemifloxacin mesylate	133%
Danofloxacin	120%
Enrofloxacin	100%
Ciprofloxacin	100%
Moxifloxacin	61%
Norfloxacin	53%
Flumequine	29%
Levofloxacin	20%
Oxolinic acid	10%
Ofloxacin	10%
Marbofloxacin	5%

12. REFERENCES

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SYMBOLS USED WITH DEMEDITEC ASSAYS

Symbol	English	Deutsch	Francais	Espanol	Italiano
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conforme aux normes européennes	Conformidad europea	Conformità europea
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las Instrucciones	Consultare le istruzioni per l'uso
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Ussage Diagnostic in vitro	Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Référence	Número de catálogo	No. di Cat.
LOT	Lot. No. / Batch code	Chargen-Nr.	No. de lot	Número de lote	Lotto no
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Note warnings and precautions	Warnhinweise und Vorsichtsmaßnahmen beachten	Avertissements et mesures de précaution font attention	Tiene en cuenta advertencias y precauciones	Annoti avvisi e le precauzioni
	Storage Temperature	Lagerungstemperatur	Temperature de conservation	Temperatura de conservacion	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
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(888) 523 1246