RIDASCREEN® Chinolone/Quinolones

Enzymimmunoassay zur quantitativen Bestimmung von Chinolonen

Enzyme immunoassay for the quantitative analysis of quinolones

Art. No.: R3113

In vitro Test
Lagerung bei 2 - 8 °C
Storage at 2 - 8 °C

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RIDASCREEN® Chinolone/Quinolones

Brief information

RIDASCREEN® Chinolone/Quinolones (Art. No.: R3113) is a competitive enzyme immunoassay for the quantitative analysis of quinolones in egg, beef, pork, sheep, chicken, turkey, fish and shrimps.
All reagents required for the enzyme immunoassay - including standards - are contained in the test kit.
The test kit is sufficient for 96 determinations (including standards).
A microtiter plate spectrophotometer is required for quantification.

Sample preparation:	egg, shrimps: homogenization, extraction, centrifugation meat, fish: homogenization, extraction, centrifugation, dilution
Time requirement:	sample preparation (for 10 samples) ..... approx. 30 min test implementation (incubation time) ..... 1 h 15 min
Detection limit: (corresponding to the standard substance)	shrimps ..... 6 ppb fish ..... 8 ppb egg ..... 9 ppb meat ..... 10 ppb
Recovery rate: (corresponding to the standard substance)	80 - 110 %
Specificity:	The specificity of the RIDASCREEN® Chinolone/Quinolones was determined by analyzing the cross-reactivities to corresponding substances in buffer system.

Ciprofloxacin	100 %
Norfloxacin, Enrofloxacin, Marbofloxacin, Danofloxacin, Difloxacin, Flumequine, Ofloxacin	> 100 %
Sarafloxacin	43 %
Oxolinic acid	24 %

1. Intended use

RIDASCREEN® Chinolone/Quinolones is a competitive enzyme immunoassay for the quantitative analysis of quinolones in egg, beef, pork, sheep, chicken, turkey, fish and shrimps.

2. General

Gyrase inhibitors are devided into four subgroups. The majority of quinolones belong to the subgroup of fluoroquinolones, which have a fluoro-group attached at the central ring system, typically at the 6th position. The fluorquinolones belong to the so called second generation quinolones. Some of these are admitted as antibiotics in veterinary medicine for food producing animals. Fluorquinolones are broad spectrum antibiotics against a lot of bacterial species. They are used frequently in veterinary medicine especially for cattle, pigs and chicken. The usage of fluorquinolone has increased in the past years because large amounts of fluorquinolones were applied to prevent infectious diseases, especially in chicken, swine and fish/shrimp farming.
The wide use in food producing animals has generated microbial resistances. This led to an amendment of Council Regulation (EEC) No 2377/90 and in the introduction of MRLs (Maximum Residue Limits) for some fluorquinolones.

3. Test principle

The basis of the test is the antigen-antibody reaction. The microtiter wells are coated with capture antibodies directed against anti-quinolone antibodies. Standards or sample solution, ciprofloxacin enzyme conjugate and anti-quinolone antibodies are added. Free quinolone and ciprofloxacin enzyme conjugate compete for the quinolone antibody binding sites (competitive enzyme immuno-assay). At the same time, the anti-quinolone antibodies are also bound by the immobilized capture antibodies. Any unbound enzyme conjugate is then removed in a washing step. Substrate/chromogen is added to the wells and incubated. Bound enzyme conjugate converts the chromogen into a blue product. The addition of the stop solution leads to a color change from blue to yellow. The measurement is made photometrically at 450 nm; the absorption is inversely proportional to the quinolone concentration in the sample.

4. Reagents provided

Each kit contains sufficient materials for 96 measurements (including standard analyses). Each test kit contains:

1 x Microtiter plate with 96 wells (12 strips with 8 removable wells each)
coated with antibodies directed against anti-quinolone antibodies
6 x Standard solutions (1.3 ml each)
0 ppb (zero standard), 0.5 ppb, 1.5 ppb, 3 ppb, 6 ppb, 18 ppb
ciprofloxacin in methanolic solution
ready to use
1 x Conjugate (6 ml) ..... red cap
peroxidase conjugated ciprofloxacin
ready to use
1 x Anti-quinolone antibody (6 ml) ..... black cap
ready to use
1 x Red Chromogen Pro (10 ml) ..... brown cap
(Substrate/chromogen solution), stained red
contains tetramethylbenzidine
1 x Stop solution (14 ml) ..... yellow cap
contains 1 N sulfuric acid
1 x Washing buffer (salt)
for preparation of a 10 mM phosphate buffer, pH 7.4
contains 0.05 % Tween 20

5. Materials required but not provided

5.1. Equipment:

- Microtiter plate spectrophotometer (450 nm)
- Centrifuge
- up-side-down shaker or vortex
- graduated pipettes
- variable 20 - 200 µl and 200 - 1000 µl -micropipettes

5.2. Reagents:

- methanol
- shrimp extraction buffer:
  dilute methanol/water (70:30, v/v) 1:2 (1+1) with washing buffer (siehe 10.1.)

6. Warnings and precautions for the users

This test should be performed only by trained laboratory staff. The instructions for performing the test must be followed strictly.

The stop solution contains 1 N sulfuric acid (R36/38, S2-26).

7. Storage instructions

Store the kit at 2 - 8 °C (35 - 46 °F). Do not freeze any test kit components.

Return any unused microwells to their original foil bag, reseal them together with the desiccant provided and further store at 2 - 8 °C (35 - 46 °F).

The substrate/chromogen solution is light sensitive, therefore, avoid exposure to direct light.

No quality guarantee is accepted after the expiration date on the kit label.

Do not interchange individual reagents between kits of different lot numbers.

8. Indication of instability or deterioration of reagents

- any bluish coloration of the reddish substrate/chromogen solution prior to test implementation
- a value of less than 0.6 absorbance units ($A_{450\ nm} < 0.6$) for the zero standard

9. Preparation of Samples

The samples should be stored in a cool place.

9.1. Egg

- homogenize the sample
- use 1 g of the homogenized sample and add 9 ml methanol/water (35:65, v/v)
- mix vigorously for 10 min (with up-side-down shaker or vortex)
- centrifuge: 10 min / 4000 g / room temperature (20 - 25 °C / 68 - 77 °F)
- use 50 µl of the supernatant per well in the test

9.2. Beef, pork, sheep, chicken, turkey, fish

- homogenize the sample
- use 1 g of the homogenized sample and add 4 ml methanol/water (70:30, v/v)
- mix vigorously for 10 min (with up-side-down shaker or vortex)
- centrifuge: 10 min / 4000 g / room temperature (20 - 25 °C / 68 - 77 °F)
- dilute the supernatant 1:2 (1+1) with washing buffer (see 10.1.)
- use 50 µl per well in the test

9.3. shrimps

- homogenize the sample
- use 1 g of the homogenized sample and add 4 ml shrimp extraction buffer (see 5.2.)
- mix vigorously for 10 min (with up-side-down shaker or vortex)
- centrifuge: 10 min / 4000 g / room temperature (20 - 25 °C / 68 - 77 °F)
- use 50 µl of the supernatant per well in the test

10. Test implementation

10.1. Preliminary comments

Bring all reagents to room temperature (20 - 25 °C / 68 - 77 °F) before use.

A PBS-Tween buffer is needed as **washing buffer**, please use the washing buffer salt (pouch) contained in the kit (see 4.). Dissolve the total content of the pouch in one liter of distilled water. The ready to use washing buffer expires after approx. 4 - 6 weeks at 2 - 8 °C (36 - 46 °F).

Alternatively: Dissolve the contents of the pouch in 100 ml of distilled water to obtain a 10fold concentrated washing buffer. This solution expires after approx. 8 - 12 weeks, stored at room temperature (20 - 25 °C / 68 - 77 °F). Use one part of this concentrate and dissolve with 9 parts of distilled water to obtain the ready to use washing buffer.

10.2. Test procedure

Carefully follow the recommended washing procedure. Do not allow microwells to dry between working steps.

1. Insert a sufficient number of wells into the microwell holder for all standards and samples to be run in duplicate. Record standard and sample positions.
2. Add 50 µl of each standard solution or prepared sample to separate duplicate wells. Use a new pipette tip for each standard or sample.
3. Add 50 µl of the enzyme conjugate solution (red cap) to the bottom of each well.
4. Add 50 µl of the antibody solution (black cap) to each well, mix gently by shaking the plate manually and incubate for 1 h in a refrigerator (2 - 8 °C / 35 - 46 °F).
5. Pour the liquid out of the wells and tap the microwell holder upside down vigorously (three times in a row) against absorbent paper to ensure complete removal of liquid from the wells. Fill all the wells with 250 µl washing buffer (see 10.1.) and pour out the liquid again. Repeat two more times.
6. Add 100 µl of substrate/chromogen (brown cap) to each well. Mix gently by shaking the plate manually and incubate for 15 min at room temperature (20 - 25 °C / 68 - 77 °F) in the dark.
7. Add 100 µl of the stop solution (yellow cap) to each well. Mix gently by shaking the plate manually and measure the absorbance at 450 nm. Read within 30 minutes after addition of stop solution.

11. Results

A special software, the RIDA®SOFT Win (Art. No. Z9999), is available for evaluation of the RIDASCREEN® enzyme immunoassays.

The course of the standard curve is shown in the Quality Assurance Certificate enclosed in the test kit.

Remark for the calculation without software:

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

The zero standard is thus made equal to 100 % and the absorbance values are quoted in percentages. The values calculated for the standards are entered in a system of coordinates on semilogarithmic graph paper against the quinolone concentration [µg/kg].

In order to obtain the quinolone concentration in µg/kg (ppb) actually contained in a sample, the concentration read from the calibration curve must be further multiplied by the corresponding dilution factor. When working in accordance with the regulation stated, the dilution factors are as follows:

shrimps	5
egg	10
meat, fish	10