IMMUNOMART

VERSION 1.01

QUINOLONES ELISA TEST KIT

MANUAL

ELISAKITS.ONLINE

By Immunomart

Quinolones ELISA Test Kit

Catalogue Number. IP100015

Principle

This test kit is based on the competitive enzyme immunoassay for the detection of Quinolones in samples. The coupling antigens are pre-coated on the micro-well stripes. The Quinolones in the sample and the coupling antigens pre-coated on the micro-well stripes compete for the anti- Quinolones antibodies. After the addition of the enzyme conjugate, the TMB substrate is added for coloration. The optical density (OD) value of the sample has a negative correlation with the Quinolones in the sample. This value is compared to the standard curve and the Quinolones concentration is subsequently obtained.

Technical specifications

Sensitivity: 1 ppb
Incubation Temperature: 25℃
Incubation Time: 30min—15min
Detection limit: Enrofloxacin (ENR) 1ppb, Ciprofloxacin (CIF) about 1ppb , Ofloxacin (OFL) about 1ppb , Danofloxacin (DAN) about 1ppb, Norfloxacin (NOR) about 0.5ppb, Lomefloxacin (LOM) about 1ppb, Pefloxacin (PEF) about 0.5ppb , Enoxacin (ENO) about 1ppb , Aosuo Li acid (OXO) about 1ppb, Fluoroquinolone acid (FLU) about 1ppb, Ma Paul Sand Star (MAR) about 1ppb , Ammonia difloxacin (AMI) about 1ppb , That difloxacin (NAD) about 2ppb
Cross-reaction rate: Enrofloxacin (ENR) 100%, Ciprofloxacin (CIF) 92.88%, Ofloxacin (OFL) 98.86%, Aosuo Li acid (OXO) 116.86%, Danofloxacin (DAN) 102.63%, Norfloxacin (NOR) 148.65%, Lomefloxacin (LOM) 86.24%, Pefloxacin (PEF) 156.60%, Enoxacin (ENO) 98.97%, Fluoroquinolone acid (FLU) 96.73% , Ma Paul Sand Star (MAR) 110.63%, Ammonia difloxacin (AMI) 98.86%, That difloxacin (NAD) 56.81%
Recovery rate: Tissue 80±15%, Serum 80±15%, Honey 75±15%

Components

1	Micro-well strips	12 strips with 8 removable wells each	
2	6× standard solution (1mL each)	0 ppb, 1 ppb, 3 ppb, 9 ppb, 27 ppb and 81 ppb	
3	Enzyme conjugate	7ml	red cap
4	Antibody working solution	7ml	blue cap
5	Substrate A	7ml	white cap
6	Substrate B	7ml	black cap
7	Stop solution	7ml	yellow cap
8	20× concentrated washing buffer	40ml	white cap
9	2× concentrated redissolving solution	50ml	transparent cap

Materials required but not provided

Equipments: microplate reader, printer, homogenizer, nitrogen-drying device, vortex, centrifuge, measuring pipets, balance (a sensibility reciprocal of 0.01 g)
Micropipettors: single-channel 20-200 µL, 100-1000 µL, and multi-channel 250 µL;

Reagents: HCl, Methylene chloride, Acetonitrile (CH_3CN), N-hexane, $Na_2HPO_4 \cdot 12H_2O$, $NaH_2PO_4 \cdot 2H_2O$, Heparin sodium

Sample pre-treatment

Instructions

1) This test kit can detect tissue sample: animal tissue, poultry, aquatic. Eg: Chicken, duck, bovine, rabbit, fish, shrimp etc.
2) Only the disposable tips can be used for the experiments and the tips must be changed when used for absorbing different reagents;
3) Before the experiment, each experimental utensil must be clean and should be re-cleaned if necessary, in order to avoid the contamination that interferes with the experimental results.

Solution preparation before sample pre-treatment
1) 0.1 M HCl: 860µl HCl (36%) + deionized water 100 mL.
2) Acetonitrile (CH_3CN) -Methylene chloride mixing solution
$V_{acetonitrile}$-$V_{methylene\ chloride}$ = 1 : 4
3) pH7.2 0.02M PB buffer: dissolve 5.16 g $Na_2HPO_4 \cdot 12H_2O$ + 0.87 g $NaH_2PO_4 \cdot 2H_2O$ in the deionized water to 1 L.
4) Acetonitrile (CH_3CN)- Methylene chloride- 0.1 M HCl mixing solution
100ml Acetonitrile (CH_3CN) -Methylene chloride mixing solution ($V_{acetonitrile}$-$V_{methylene\ chloride}$ = 4 : 1), add 5ml 0.1 M HCl solution.
5) The 2× concentrated redissolving solution is diluted with deionized water at 1:1 (1 mL concentrated redissolving solution + 1 mL deionized water), used for the sample redissolving.

Samples preparation

a) Tissues (chicken/liver, pork/liver, fish, shrimp etc.)

1. Weigh 2.0 ± 0.05 g of the homogenized tissue sample into 50 ml centrifuge tube
2. Add 8 ml of the Acetonitrile (CH_3CN) -Methylene chloride mixing solution, shake for 5 min, centrifuge at above 4000 r/min at 15 ℃ for 10 min
3. Take 4 ml the clear organic phase (upper layer) into a dry tube, blow to dry with nitrogen or air completely by rotary evaporation at 56 ℃
4. Dissolve the dry residues in 1 mL of the diluted redissolving solution, add 1 mL N-hexane, mix for 30 seconds; centrifuge at above 4000 r/min at 15℃ for 5 min.
5. Remove the upper layer, take 50µl lower layer solution for further analysis.

Fold of dilution of the sample: 1

b) Serum

1. Use centrifuge tube with heparin sodium (20-30 unit/ml blood) to collect chicken blood sample (Suggestion: blood collection syringes are recommended rinsing with heparin).Place the blood sample in the room temperature for 1 hour. After obtain plasma, centrifuge at above 4000r/min at 15 ℃ for 10 min, take out 1 ml plasma.
2. Add CH_3CN (without water) 4 ml , mix up-and-down thoroughly for 5 min, centrifuge at above 4000r/min at 15 ℃ for 10 min.
3. Move the clear supernatant (upper layer) to another centrifuge tube, add 2ml 0.02M PB buffer, mix evenly.
4. Add 5 ml Methylene chloride, mix evenly for 5 min, centrifuge at above 4000r/min at

15 ℃ for 10 min, remove the upper layer, take the lower organic phase to dry bottle(clear without impurities), blow to dry with nitrogen or air completely by rotary evaporation at 50 ℃

5. Dissolve the dry residues in 1 mL of the diluted redissolving solution, add 1 mL N-hexane, mix for 30 seconds; centrifuge at above 4000 r/min at 15℃ for 5 min.

6. Absorb out lightly the upper and middle layer white impurities, take lower phase 100μl, add 100μl diluted redissolving solution, mix for 30s.

7. Take 50μl solution for further analysis.

Fold of dilution of the sample: 2

c) Honey

1. Weigh 2.0±0.05g honey sample into 50ml centrifuge tube, add 8 ml Acetonitrile (CH_3CN)- Methylene chloride- 0.1 M HCl mixing solution, shake fully for 3min, centrifuge at above 4000 r/min at 15 ℃ for 10 min.
2. Take 2ml the supernatant (upper layer), blow to dry with nitrogen or air at 56 ℃.
3. Add 1 mL the diluted redissolving solution, shake fully for 1min.
4. Take 50 μL for further analysis

Fold of dilution of the sample: 2

ELISA procedures

Instructions

1. Bring all reagents and micro-well strips to the room temperature (20-25 ℃) before use;
2. Return all reagents to 2-8 ℃ immediately after use;
3. The reproducibility of the ELISA analysis, to a large degree, depends on the consistency of plate washing. The correct operation of plate washing is the key point in the procedures of ELISA;
4. For the incubation at constant temperatures, all the samples and reagents must avoid light exposure, and each microplate should be sealed by the cover membrane.

Operation procedures

1. Take out all the necessary reagents and place at the room temperature (20-25 ℃) for at least 30min. Note that each reagent must be shaken to mix evenly before use;
2. Take the required micro-well strips and plate frames. Re-sealed the unused microplate, stored at 2-8 ℃;
3. Solution preparation: dilute 40 mL of the 20× concentrated washing buffer with the deionized water at 1:19 (1 part of 20× concentrated washing buffer + 19 parts of deionized water), or prepare as quantity needed;
4. Numbering: number the micro-wells according to samples and standard solution; each sample and standard solution should be performed in duplicate; record their positions;
5. Add 50 μL of the sample or standard solution to separate duplicate wells, add 50 μL of enzyme conjugate, then add 50 μL of the antibody working solution into each well. Mix by shaking gently, seal the microplate with the cover membrane, and incubate at 25℃ for 30min;
6. Wash the microplate with the washing buffer at 250 μL/well for four to five times.;soak the well with the washing buffer for 15-30 sec, flap to dry with absorbent paper (if there are the bubbles after flapping, cut them with the clean tips);
7. Coloration: add 50 μL of the substrate A solution and 50 μL of the B solution into each well. Mix by shaking gently, and incubate at 25 ℃ for 15 min in the dark for coloration;

8. Determination: add 50 µL of stop solution into each well. Mix by shaking gently. Set the wavelength of the microplate reader at 450 nm to determine the OD value. (recommend to read the OD value at the dual-wavelength 450/630 nm within 5 min) .

Result judgment

There are two methods to judge the results; the first one is the rough judgment, while the second is the quantitative determination. Note that the OD value of the sample has a negative correlation with the content of Quinolones in the sample.

Qualitative determination

The concentration range (ng/mL) obtained from the comparison the average OD value of the sample with that of the standard solution. Assuming that the OD value of the sample Ⅰ is 0.238, and that of the sample Ⅱ is 0.946, the OD value of standard solutions is: 1.845 for 0 ppb, 1.542 for 1 ppb, 1.130 for 3 ppb, 0.635 for 9 ppb, 0.326 for 27 ppb ,0.156 for 81 ppb, accordingly the concentration range of the sample Ⅰ is 27 to 81 ppb, and that of the sample Ⅱ is 3 to 9 ppb. (multiplied by the corresponding dilution fold)

Quantitative determination

The mean values of the absorbance values obtained for the percentage of the average OD value (B) of the sample and the standard solution divided by the OD value (B0) of the first standard solution (0 standard) and subsequently multiplied by 100%, that is,

Percentage of absorbance value = $(B / B_0) \times 100\%$

B—the average OD value of the sample or the standard solution
B_0—the average OD value of the 0 ng/mL standard solution

Draw the standard curve with the absorption percentages of the standard solutions and the semilogarithm values of the Quinolones standard solutions (ng/mL) as Y- and X-axis, respectively. Read the corresponding concentration of the sample from the standard curve by incorporating its absorption percentage into the standard curve. The resulting value is subsequently multiplied by the corresponding dilution fold, finally obtaining the Quinolones concentration in the sample.
Using the professional analyzing software of this kit will be more convenient for the accurate and rapid analysis of a large amount of samples. (Please contact us for this software)

Precautions

1. The room temperature below 25 ℃ or the temperature of the reagents and the samples being not returned to the room temperature (20-25 ℃) will lead to a lower standard OD value.
2. Dryness of the microplate in the washing process will be accompanied by the situations including the non-linear standard curves and the undesirable reproducibility; So continue to next step immediately after washing.
3. Mix evenly, otherwise there will be the undesirable reproducibility.
4. The stop solution is the 2 M sulfuric acid solution, avoid contacting with the skin.
5. Do not use the kit exceeding its expiry date. The use of diluted or adulterated reagents from the kits will lead to the changes in the sensitivity and the detecting OD values. Do not exchange the reagents from the kits of different lot numbers to use.
6. Put the unused microplate into an auto-sealing bag to re-seal it. The standard substance and the colourless color former is light sensitive, and thus they cannot be directly exposed to the light.

7. Discard the colouration solution with any color that indicates the degeneration of this solution. The detecting value of the 0 standard solution of less than 0.5 (A450 nm< 0.5) indicates its degeneration.
8. The optimum reaction temperature is 25 ℃, and too high or too low temperatures will result in the changes in the detecting sensitivity and OD values.

Storage: store at 2-8 ℃, not frozen.
Expiry date: 12 months; date of production is on box.