QUALICHEK™ AOZ (Furazolidone Metabolite) ELISA

REF : KBFP1008

Designed and Developed as per AOAC Official Methods of Analysis Guidelines (Method 971.22)

ISO9001:2016 ISO13485:2016

Ver 1.1

RUO

Quantitative testing of AOZ (Furazolidone Metabolite) in samples such as Tissue, Liver, Milk (liquid/powder), Egg powder, Honey, Casing, Fodder, Fish, Shrimp, etc.

RUO	**For Research Use Only**	**REF**	**Catalog Number**
	Store At	**LOT**	**Batch Code**
	Manufactured By		**Biological Risk**
	Expiry Date		**Consult Operating Instructions**

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REF KBFP1008 Σ 96 tests

KRISHGEN BioSystems
For US/Europe Customers: toll free +1(888)-970-0827 | tel +1(562)-568-5005
For Asia/India Customers: +91(22)-49198700
Email: sales@krishgen.com | http://www.krishgen.com

Introduction:

The ELISA kits are used for assessing the specific biomarker in samples analytes which may be such as Tissue, Liver, Milk (liquid/powder), Egg powder, Fodder, Honey, Casing, etc as validated with the kit. The kit employs a competitive ELISA technique..

Intended Use:

The QUALICHECK™ AOZ (Furazolidone Metabolite) ELISA is used for quantitative testing of AOZ (Furazolidone Metabolite) in sample, such as Tissue, Liver, Milk (liquid/powder), Egg powder, Fodder, Honey, Casing, Fodder, Fish, Shrimp, etc.

Principle:

The method employs the quantitative competitive enzyme immunoassay technique. The microtiter plate is pre-coated with coupled antigen. During the reaction, AOZ (Furazolidone Metabolite) in the samples or standard competes with coupled antigen on the microplate for sites of anti-AOZ (Furazolidone Metabolite) antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each microtiter plate well, and TMB substrate is added. Color development is stopped by addition of stop solution. Absorbance is measured at 450 nm(reference wavelength 630 nm). There is a negative correlation between the OD value of samples and the concentration of AOZ (Furazolidone Metabolite).

Materials Provided:

1. AOZ (Furazolidone Metabolite) Antigen Coated Microtiter plate - 1x 96 wells
2. AOZ (Furazolidone Metabolite) Standard, (6 x 1 ml) - 0, 0.05, 0.15, 0.45, 1.35, 4.05 ppb
3. Derivatization Reagent – 10 ml
4. HRP Conjugate – 5.5 ml
5. Antibody Working Solution – 5.5 ml
6. TMB Substrate - 12 ml
7. (20X) Wash Buffer - 25 ml
8. Stop Solution - 12 ml
9. (2X) Reconstitution Buffer – 50 ml
10. Instruction Manual - 1 no

Materials to be provided by the End-User:

1. Microplate reader
2. Printer
3. Homogenizer
4. Nitrogen evaporators
5. Water bath
6. Vortex mixer
7. Centrifuge
8. Graduated pipette
9. Balance (sensibility 0.01 g)
10. Single channel (20-200 µL, 100-1000 µL)
11. Multichannel (30-300 µL)
12. Ethyl acetate
13. N-hexane
14. NaOH
15. HCl
16. $K_2HPO_4 \cdot 3H_2O$
17. $ZnSO_4 \cdot 7H_2O$
18. $Na_2Fe(CN)_5NO \cdot 2H_2O$

Handling/Storage:

1. All reagents should be stored at 2ºC to 8ºC for stability.
2. All the reagents and wash solutions should be used within 12 months from manufacturing date.
3. Before using, bring all components to room temperature (18-25ºC). Upon assay completion ensure all components of the kit are returned to appropriate storage conditions.
4. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.
2. For research use only

Sample Preparation:

Bring all reagents and samples to room temperature before use.
Open the microplate reader in advance, preheat the instrument, and set the testing parameters.

Sample Pre-treatment Notice:

Experimental apparatus should be clean; use disposable pipette tips to avoid cross-contamination during the experiment.

Solution preparation
Please prepare solution according to the number of samples. Don't use up all components in the kit at once!

Solution 1: 0.36 M Na_2Fe (CN)5 NO •$2H_2O$ Solution (for milk and milk powder sample).
Dissolve 10.7 g of **$Na_2Fe(CN)_5NO•2H_2O$** to 100 mL with deionized water.

Solution 2: 1.04 M $ZnSO_4$ Solution (for milk and milk powder, egg powder sample)
Dissolve 29.8 g of **$ZnSO_4•7H_2O$** to 100 mL with deionized water, mix fully.

Solution 3: 0.1 M K_2HPO_4 Solution
Dissolve 11.4 g of **$K_2HPO_4•3H_2O$** to 500 mL with deionized water, mix fully.

Solution 4: 1 M HCl Solution
Dilute 8.6 mL of HCl to 100 mL with deionized water, mix fully.

Solution 5: 1 M NaOH Solution
Dissolve 4 g of **NaOH** to 100 mL with deionized water.

Solution 6: Reconstitution Buffer
Dilute the (**2X) Reconstitution Buffer** with deionized water (2X Reconstitution Buffer (V): Deionized water (V)=1:1) .The Reconstitution buffer can be store at 4℃ for a month.

Solution 7: Wash Buffer
Dilute **20X Wash Buffer** with deionized water. (20X Wash Buffer (V): Deionized water (V) = 1:19).

Sample pretreatment procedure
Pretreatment of milk sample:
(1) Take 5 mL of milk into 50 mL centrifuge tube, add 250 µL of **0.36 M $Na_2Fe(CN)_5NO•2H_2O$ Solution** (Solution 1) and vortex for 30s, then add 250 µL of **1.04 M $ZnSO_4$ Solution** (Solution 2) and vortex for 30s, centrifuge at 4000 rpm for 10min at 15℃. If a refrigerated centrifuge is not available, chill sample to approx. 15 °C prior to centrifugation.
(2) Take 1.1 mL of supernatant to another centrifuge tube, add 4 mL of deionized water, 0.5 mL of **1 M HCl Solution** (Solution 4) and 100 µL of **Derivatization Reagent**, vortex for 5 min.
(3) Incubate overnight at 37℃(about 16 hours) or incubate in water bath at 50℃ for 3 hours (the effect of stratification will be affect when more than 50℃).

(4) Add 5 mL of **0.1 M K_2HPO4 Solution** (Solution 3), 0.4 mL of **1 M NaOH Solution** (Solution 5) and 5 mL of **Ethyl acetate**, vortex for 5 min.
(5) Centrifuge at 4000 rpm at room temperature for 10 min.
(6) Take 2.5 mL of upper liquid to another centrifuge tube, dry at 50-60℃ with nitrogen evaporators or water bath. Dissolve the residual with 1 mL **N-hexane**, add 1 mL of **Reconstitution Buffer** (Solution 6) and vortex for 30s. Centrifuge at 4000 rpm at room temperature for 10 min.
(7) Discard the upper n-hexane, take 50 µL lower liquid for analyze
Note: Sample dilution factor: 2, detection limit: 0.1 ppb

Pretreatment of milk powder, egg powder sample:
(1) Weigh 1±0.05 g of sample into 50 mL centrifuge tube, add 4 mL of deionized water, 0.5 mL of **1 M HCl Solution** (Solution 4) and 100 µL of **Derivatization Reagent**, vortex for 5 min.
(2) Incubate overnight at 37℃(about 16 hours) or incubate with water bath at 50℃ for 3 hours (the effect of stratification will be affect when more than 50℃).
(3) Add 250 µL of **0.36 M $Na_2Fe(CN)_5NO \cdot 2H_2O$ Solution** (Solution 1), vortex for 30s, then add 250 µL of **1.04 M ZnSO4 Solution** (Solution 2), vortex for 30s centrifuge at 4000 rpm at 15℃ for 10 min. If a refrigerated centrifuge is not available, chill sample to approx 15°C prior to centrifugation.
(4) Take all supernatant to another centrifuge tube, add 5mL of **0.1 M K_2HPO_4 Solution** (Solution 3), 0.4 mL of **1 M NaOH Solution** (Solution 5) and 5 mL of **Ethyl acetate**, vortex for 5 min.
(5) Centrifuge at 4000 rpm at room temperature for 10 min.
(6) Take 2.5 mL of upper liquid to another centrifuge tube, dry at 50-60℃ with nitrogen evaporators or water bath.
(7) Dissolve the residual with 1mL **N-hexane**, add 1 mL of **Reconstitution Buffer** (Solution 6) and vortex for 30s. Centrifuge at 4000 rpm at room temperature for 10 min.
(8) Discard the upper n-hexane, take 50 µL of lower liquid for analyze.
Note: Sample dilution factor: 2, detection limit: 0.1 ppb

Pretreatment of honey, muscle (livestock, fish, shrimp), liver, feed, egg sample :
(1) Remove fat from sample (except feed, honey and eggs). Homogenize the representative sample with a homogenizer and mix fully.
(2) Weigh 1±0.05 g of homogenate sample into 50 mL centrifuge tube, add 4 mL of deionized water, 0.5 mL of **1 M HCl Solution** (Solution 4) and 100 µL of **Derivatization Reagent**, vortex for 5min.
(3) Incubate overnight at 37℃ (about 16 hours) or incubate in water bath at 50℃ for 3 hours (the effect of stratification will be affect when more than 50℃).
(4) Add 5 mL of **0.1 M K_2HPO_4 Solution** (Solution 3), 0.4 mL of **1 M NaOH Solution** (Solution 5) and 5 mL of **Ethyl acetate**, vortex for 5 min.
(5) Centrifuge at 4000 rpm at room temperature for 10 min.
(6) Take 2.5 mL of upper liquid to another centrifuge tube, dry at 50-60℃ with nitrogen evaporators or water bath.
(7) Dissolve the residual with 1 mL **N-hexane**, add 1 mL of **Reconstitution Buffer** (Solution 6) and vortex for 30s. Centrifuge at 4000 rpm at room temperature for 10 min.
(8) Discard the upper n-hexane, take 50 µL lower liquid for analyze
Note: Sample dilution factor: 2, detection limit: 0.1 ppb

Assay Procedure:
1. Prepare all reagents and samples as directed in previous sections.

2. Take out required wells and rack and put back the rest into aluminum foil bag and seal it. Store at 2-8°C.

3. Add **50 ul Standard or Sample** into wells in sequence.

4. Add **50 ul Antibody Working Solution** into each well in sequence.

5. Add **50µL HRP-conjugate** into each well in sequence. Seal plate with adhesive strip and shake plate for 30s. Incubate at 25°C for 45 minutes.

6. Take out microtiter plate and remove adhesive strip and then spin-dry liquid in wells on absorbent paper. Then wash plate with diluted wash buffer by four times. Every time, soak each well with 250uL diluted wash buffer for 30s, and then spin-dry liquid in wells. If you use squirt bottle, multi-channel pipette, manifold dispenser, or autowasher, let it stand for 15~30 seconds, because complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining wash buffer by aspirating or decanting. Invert the plate and blot it against absorbent paper.

7. Add **100uL TMB Substrate** into each well in sequence, then shake it softly for a while. Later, seal plate with adhesive strip again and then incubate at 25°C for 15 minutes.

8. Add **100µL Stop Solution** into each well in sequence so as to stop reaction (Blue turns Yellow). Gently tap the plate to ensure thorough mixing. The sequence of adding Stop Solution shall be the same sequence of adding TMB Substrate. In order to guarantee result accuracy, you shall add Stop Solution as soon as TMB Substrate reaction time reaches to fifteen minutes.

9. Read OD value with Microplate Reader at 450nm/630nm. Please read data in five minutes after adding Stop Solution.

Calculation of Results:

There are two methods to judge result: the first one (A) is the rough judgment, while the second (B) is quantitative determination. Please note that OD value has negative correlation with AOZ in the sample.

A: Compare the sample average absorbance values with standards values, the AOZ concentration in the samples can be concluded.

B: The mean values of absorbance values obtained for the standards and the samples, are divided by the absorbance value of the first standard (zero standard) and multiplied by 100%. The zero standard is thus made equal to 100% and the absorbance values are quoted in percentages.

Absorbance (%) = A/A_0 x 100%

A - the average absorbance value of the sample or standard
A_0 - the average absorbance value of the 0 ppb standard

To draw a standard curve: Take the absorbance value of standards as y-axis, semi-logarithmic of the concentration of the AOZ standards solution (ppb) as x-axis.

The AOZ concentration of each sample (ppb), which can be read from the calibration curve, is multiplied by the corresponding Dilution factor of each sample followed, and then you get actual concentration of AOZ in sample.

Notes:

1. Final test results will be closely related to validity of products, operation skills of end users and test environment.
2. Do test smoothly. Do not put aside microplate without moving to next step for a long time, as it may cause dry wells, degeneration of antibody or antigen, and finally bad result.
3. It is necessary to cover adhesive strip during incubation to prevent sample evaporation or contamination. Do not keep wells dry during test. Observe incubation temperature and time and adjust it timely. Do not open incubator too frequently to affect temperature.
4. Insufficient washing will result in poor precision and falsely elevated absorbance reading.
5. Observe the change of color after adding TMB Substrate (e.g. observation once every 10minutes). TMB Substrate shall change color from colorless or light blue to gradations of blue. If the color is too deep, add Stop Solution in advance to avoid excessively strong reaction which will result in inaccurate OD value.
6. TMB Substrate is easily contaminated. It shall remain colorless or light blue until it is added into plate. Store it avoiding light. Discard it if you see any color that indicates degeneration of solution. When absorbance value of standard solution S1 is less than 0.5, it indicates degeneration.
7. Wells turn green indicate that Stop Solution has not mixed thoroughly with TMB Substrate.
8. Best reaction temperature is 25°C. Too high or too low reaction temperature will influence OD value and sensitivity.

Performance Characteristics of the Kit:
Sensitivity: 0.05 ppb (ng/mL)

Reaction mode (Incubation time and temperature): 25ºC; 45 min, 15 min

Detection limit: Muscle, Liver, Honey, Milk, Egg ---0.1 ppb; Milk powder, Egg powder, Feed---0.1 ppb

Cross-reactivity: AOZ(3-Amino-2-oxazolidinone)---100%, AMOZ, AHD, SEM---＜0.1%

Sample recovery rate: Muscle, Liver---80%±25%, Honey, Milk ---75%±15%,
Milk powder, Egg powder, Feed, Egg---85%±25%

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