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Furazolidone ELISA Kit

07/16

(Catalog # K4231-100, 100 assays, Store at 4°C)

I. Introduction:

Furazolidone is a nitrofuran antibacterial agent. Nitrofurans are recognized by FDA as mutagens/carcinogens, and can no longer be used since 1991, and it has been prohibited from the use in food-producing animals in the European Union since 1997. BioVision's Furazolidone ELISA kit is a competitive ELISA assay for the quantitative measurement of human Furazolidone in animal tissues or food. The density of color is proportional to the amount of human Furazolidone captured from the samples.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of Furazolidone.
Detection Range: 0.02 – 1.62 ppb (ng/ml)
Sensitivity: < 0.02 ppb
Detection Limit: 0.1 ppb for Fish and Shrimp, 0.4 ppb for Tissue, Liver, Honey, Milk, Milk powder, Egg powder.

III. Specificity:

Universal
Cross reaction rate: ＜0.1% for AMOZ and AHD

IV. Sample Type:

Fish, shrimp, Tissue, Liver, Honey, Milk, Milk powder, Egg powder

V. Kit Contents:

Components	K4231-100	Part No.	Cap Color
Micro ELISA Plate	8 X 12 strips	K4231-100-1	-
Standards (0, 0.02, 0.06, 0.18, 0.54, 1.62 ppb)	1.0 ml X 6	K4231-100-2	-
High standard (100 ppb)	1 ml	K4231-100-3	Red
Derivatization reagent	1 ml	K4231-100-4	Black
Antibody working solution	5.5 ml	K4231-100-5	Blue
Enzyme conjugate	5.5 ml	K4231-100-6	Red
Substrate A solution	6 ml	K4231-100-7	White
Substrate B solution	6 ml	K4231-100-8	Black
Stop Solution	6 ml	K4231-100-9	Yellow
Concentrated Wash Solution (20X)	40 ml	K4231-100-10	White
Concentrated Redissolving solution (2X)	50 ml	K4231-100-11	Yellow
Plate sealers	1	K4231-100-12	-

VI. User Supplied Reagents and Equipment:

- Reagents: ethyl acetate, N-hexane, NaOH, HCl, potassium hydrogen phosphate anhydrous ($K_2HPO_4 \cdot 3H_2O$), potassium nitroferrocyanide ($K_2Fe(CN)_5(NO) \cdot 2H_2O$) and $ZnSO_4 \cdot 7H_2O$
- Microplate reader capable of measuring absorbance at 450 nm
- Precision pipettes with disposable tips
- Distilled or deionized water
- Clean eppendorf tubes and centrifuge tube for preparing standards or sample dilutions
- Absorbent paper

VII. Storage and Handling:

The entire kit may be stored at 4°C for up to 12 months from the date of shipment.

VIII. Reagent Preparation:

Note: Read the entire protocol before starting.
Before using the kit, spin tubes and bring down all components to the bottom of tubes.

1. **Wash Buffer:** Dilute 40 ml of the concentrated washing buffer with the distilled or deionized water to 800ml (or just to the required volume) for using.
2. **Sample Preparation**:

Note: Samples to be used within 5 days may be stored at 4°C, otherwise samples must be stored at -20°C (≤1 month) or -80°C (≤2 months) to avoid loss of bioactivity and contamination. Avoid multiple freeze-thaw cycles.

- **Milk:** Take 5 ml milk into centrifuge tube, add 250 µl 0.36M potassium nitroferrocyanide solution (0.119 g/ml), oscillate for 30 sec, then add 250 µl 1.04 M ZnSO4•7H2O solution (0.298 g/ml), oscillate for 30 sec, centrifuge at 4000 r/min at 15°C for 10 min. Take 1.1 ml supernatant, add 4 ml ddH_2O, 0.15ml 1M HCl, and 100µl Derivatization reagent, and oscillate 5 min. Incubate in water at 37°C over night or Incubate at 50°C for 3 hour. Add 5 ml 0.1 M K_2HPO_4 solution, 0.4 ml 1 M NaOH solution, and 5 ml ethyl acetate to the tube, oscillate vigorously for 5 min. Centrifuge at 4000 rpm at room temperature for 10 min. Transfer 2.5 ml supernatant to another centrifuge

tube and blow dry at 50 - 60°C with nitrogen or air. Add 1 ml N-hexane to dissolve the dried residue, and then add 1 ml redissolving solution, oscillate 30 sec, centrifuge at 4000 rpm at RT for 10 min. Remove the upper layer; take 50µl Lower phase for the analysis.

- **Milk powder, Egg powder**: Weight 1 g homogeneous samples into the 50 ml centrifuge tube. Add 4 ml ddH2O, 0.15 ml 1M HCl, and 100 µl Derivatization reagent and oscillate for 5 min. Incubate the samples in 37°C water bath over night or Incubate at 50°C for 3 hour. Afterward, add 250 µl of 0.36M potassium nitroferrocyanide (0.119 g/ml) and oscillate for 30 sec. Add 250 µl of 1.04 M Zinc Sulfate solution (0.298 g/ml) and oscillate for 30 sec. After all reagents are fully mixed, centrifuge samples at 4000 rpm at 15°C for 10 min. Extract all the supernatant to another centrifuge tube. Add 5 ml 0.1 M K_2HPO_4 solution, 0.4 ml 1 M NaOH solution, and 5 ml ethyl acetate to the tube, oscillate vigorously for 5 min. Centrifuge at 4000 rpm at room temperature for 10 min. Transfer 2.5 ml supernatant to another centrifuge tube and blow dry at 50 - 60°C with nitrogen or air. Add 1 ml N-hexane to dissolve the dried residue, and then add 1 ml redissolving solution, oscillate 30 sec, centrifuge at 4000 rpm at RT for 10 min. Remove the upper layer; take 50µl Lower phase for the analysis. (Dilution factor: 2)
- **Honey, Tissue, Prepared intestine, Liver, Egg**: Weight 1 g homogeneous samples into a 50ml centrifuge tube. Add 4 ml ddH_2O, 0.15 ml 1M HCl, and 100 µl Derivatization reagent, and then oscillate for 5 min. Incubate in water at 37°C overnight or Incubate at 50°C for 3 hour. Add 5 ml 0.1 M K_2HPO_4 solution, 0.4 ml 1 M NaOH solution, and 5 ml ethyl acetate to the tube, oscillate vigorously for 5 min. Centrifuge at 4000 rpm at room temperature for 10 min. Transfer 2.5 ml supernatant to another centrifuge tube and blow dry at 50 - 60°C with nitrogen or air. Add 1 ml N-hexane to dissolve the dried residue, and then add 1 ml redissolving solution, oscillate 30 sec, centrifuge at 4000 rpm at RT for 10 min. Remove the upper layer; take 50 µl Lower phase for the analysis. (Dilution factor: 2)
- **Cooked food:** Weight 1 g homogeneous samples into the 50 ml centrifuge tube. Mix thoroughly for 2 min with 4.5 ml methanol and 0.5ml ddH_2O. Centrifuge at 4000 rpm at RT for 5 min and discard supernatant. Add 5 ml acetonitrile and 5 ml N-hexane and oscillate for 2 min, centrifuge at 4000 rpm at RT for 5 min, and discard supernatant. Pipette 4 ml ddH_2O, 0.5 ml 1 M HCl and 100 µl derivatization reagent into the precipitation, oscillate 5min. Incubate in water at 37°C overnight or Incubate at 50°C for 3 hour. Add 5 ml 0.1 M K_2HPO_4 solution, 0.4 ml 1 M NaOH solution, and 5 ml ethyl acetate to the tube, oscillate vigorously for 5 min. Centrifuge at 4000 rpm at room temperature for 10 min. Transfer 2.5 ml supernatant to another centrifuge tube and blow dry at 50 - 60°C with nitrogen or air. Add 1 ml N-hexane to dissolve the dried residue, and then add 1 ml redissolving solution, oscillate 30 sec, centrifuge at 4000 rpm at RT for 10 min. Remove the upper layer; take 50µl Lower phase for the analysis.

IX. Assay Protocol:

Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay. Shake the reagent bottles if there is any crystal.
It is recommended that all standards and samples be run at least in duplicate.
A standard curve must be run with each assay.

1. Prepare all reagents, samples and standards as instructed in section VIII.
2. Add 50 µl **standards** or **samples** into marked well, then add 50 µl **HRP-conjugate** solution into each well.
3. Add 50 µl **antibody working solution** into each well. Oscillate the plate for 5 sec, cover the well and incubate for 45 min at RT (25°C).
4. Discard solution, wash plate 5 times with **1X Wash Solution**. Wash by filling each well with Wash Buffer (250 µl) using a multi-channel pipette or autowasher. Let it soak for 1 min, and then remove all residual wash-liquid from the wells. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Clap the plate on absorbent filter papers or other absorbent materials.
5. Pipette 50 µl **Substrate A solution**, then pipette 50 µl **Substrate B solution** to each well, oscillate gently for 5 sec, avoid the light preservation for 15 min at RT (Do not put any substrate back to the original container to avoid any potential contamination.)
6. Add 50 µl **Stop Solution** to each well and oscillate gently to stop the reaction. Read result at 450 nm within 10 minutes.

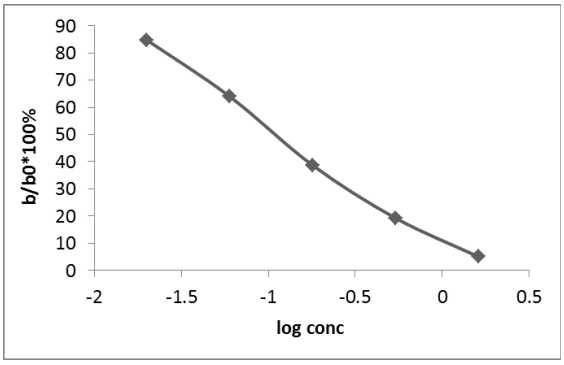
X. CALCULATION:

Percentage of absorbance value (%) = A/A_0 X 100%

A: the average (double wells) OD value of the sample or the standard solution; A_0: the average OD value of the 0 ppb standard solution.

To draw the standard curve and calculate, take absorbance percentage of standards as Y-axis, the corresponding log of standards concentration (ppb) as X-axis. Draw the standard semilog curves with X-axis and Y-axis. Take absorbance percentage of samples substitute into standard curve, then can get the corresponding concentration from standard curve; last, Multiplied by the corresponding dilution times is the actual concentration of samples.

Figure: Typical Standard Curve: These standard curves are for demonstration only. A standard curve must be run with each assay.



XI. RELATED PRODUCTS:

- Salbutamol (SALB) ELISA Kit (Cat. No. K4209-100)
- Sulfonamides residue ELISA Kit (Cat. No. K4207-100)
- Aflatoxin B1 (AFB1) ELISA Kit (Cat. No. K4208-100)
- Fluoroquinolones ELISA Kit (Cat. No. K4205-100)
- Gentamicin ELISA Kit (Cat. No. K4206-100)
- Kanamycin ELISA Kit (Cat. No. K2410-100)