Nitrofurantoin (AHD) ELISA Kit

Rev 11/21

(Catalog # E4275-100, 96 assays, Store at 4°C)

I. Introduction:

Nitrofurantoin works by slowing growth rather than killing bacteria. Nitrofurantoin is concentrated in the urine, leading to higher and more effective levels in the urinary tract than in other tissues or compartments. It works by damaging bacterial DNA, since its reduced form is highly reactive. This is made possible by the rapid reduction of nitrofurantoin inside the bacterial cell by flavoproteins (nitrofuran reductase) to multiple reactive intermediates that attack ribosomal proteins, DNA, respiration, pyruvate metabolism and other macromolecules within the cell. Nitrofurantoin exerts greater effects on bacterial cells than mammalian cells because bacterial cells activate the drug more rapidly. **Nitrofurantoin ELISA Kit** is a competitive ELISA assay for the quantitative measurement of Nitrofurantoin in tissue, liver, honey, milk, milk powder, egg, fish and shrimp. The Nitrofurantoin coupling antigen has been coated in the plate wells. During the detection, add standards or samples to the well, AHD of samples will compete with coated AHD coupling antigen to combine with antibody, after adding antibody working solution, HRP, take coloration with TMB substrate. The samples A value is an inverse relationship with AHD residue content. Lastly, compare with the standard curve. The density of color is proportional to the amount of Nitrofurantoin captured from the samples.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of Nitrofurantoin.
Detection Range: 0.02 – 1.62 ppb (ng/ml)
Sensitivity: 0.02 ppb (ng/ml)
Detection limit: 0.04 ppb for tissue, liver, honey, milk, milk powder, egg powder; 0.06 ppb for fish and shrimp

III. Sample Type:

Milk powder, milk, tissue, egg, liver, honey, fish, shrimp

IV. Kit Contents:

Components	E4275-100	Part No.	Cap Color
Micro ELISA Plate	8 X 12 strips	E4275-100-1	-
Standards (S1 – S6)	1.0 ml X 6	E4275-100-2-x	-
High standard (100 ppb)	1.0 ml	E4275-100-3	Red
Antibody working solution	5.5 ml	E4275-100-4	Blue
Enzyme conjugate	5.5 ml	E4275-100-5	Red
Substrate A solution	6 ml	E4275-100-6	White
Substrate B solution	6 ml	E4275-100-7	Black
Stop Solution	6 ml	E4275-100-8	Yellow
Concentrated Wash Solution (20X)	40 ml	E4275-100-9	White
Concentrated Redissolving solution (2X)	50 ml	E4275-100-10	Yellow
Derivatization reagent	10 ml	E4275-100-11	Black
Adhesive Membrane	1	E4275-100-12	-
Sealed bag	1	E4275-100-13	-

V. User Supplied Reagents and Equipment:

- **Reagents**: ethyl acetate, N-hexane, sodium hydroxide, thick HCl, potassium hydrogen phosphate anhydrous ($K_2HPO_4 \cdot 3H_2O$), potassium nitroferrocyanide ($K_2Fe(CN)_5(NO) \cdot 2H_2O$); Zinc Sulfate ($ZnSO_4 \cdot 7H_2O$)
- Microplate reader capable of measuring absorbance at 450 nm
- Precision pipettes with disposable tips
- Distilled or deionized water
- Nitrogen-drying device
- Clean Eppendorf tubes for preparing standards or sample dilutions
- Absorbent paper

VI. Storage and Handling:

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

VII. Reagents and Samples Preparation:

Note: Prepare reagents within 30 minutes before the experiment.
Before using the kit, spin tubes and bring down all components to the bottom of tubes.

1. **Standards:** ready to use.

Tube #	S1	S2	S3	S4	S5	S6
Concentration (ng/ml)	0	0.02	0.06	0.18	0.54	1.62

2. **Redissolving solution**: Dilute the concentrated redissolving solution 2 times with deionized water to be used for sample redissolving, it can be stored at 4 ℃ environment up to a month.
3. **Wash Buffer:** Dilute 40 ml of the concentrated washing buffer with the distilled or deionized water to 800 ml (or just to the required volume) for using.
4. **Sample Preparation**:

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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**: Mix 5 ml milk and 250 µl 0.36 M potassium nitroferrocyanide solution in a centrifuge tube and oscillate for 30 sec. Add 250 µl 1.04 M Zinc Sulfate solutions and oscillate for 30 sec, centrifuge at 4000 rpm at 15°C for 10 min. Take 1.1 ml supernatant and mix with 4 ml deionized water, 0.15 ml 1M HCl, and 100 µl derivatization reagent, and oscillate 5 min. Incubate in 37°C water bath overnight or in 50°C for 3 hours. 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. Wipe out 2.5 ml supernatant to another centrifuge tube and blow dry at 50 to 60°C with nitrogen or air. Add 1 ml N-hexane to dissolve the dried residue, and then add 1 ml redissolving solution, oscillate for 30 sec, centrifuge at 4000 rpm at room temperature for 10 min. Remove the upper N-hexane; take 50 µl Lower phase for the analysis. (Dilution times of the sample: 1:2)
- **Milk powder, Egg powder**: Weight 1 g homogeneous samples into the 50 ml centrifuge tube, pipette 4 ml deionized water, 0.15 ml 1M HCl, and 100 µl Derivatization reagent, oscillate for 5 min. Incubate in 37°C water over night (approximately 16 hours) or Incubate at 50°C for 3 hours. Add 250 µl 250µl potassium nitroferrocyanide solution, oscillate 30s, and then add 250 µl Zinc Sulfate solutions, oscillate 30 sec, centrifuge 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 to 60 ℃ 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 room temperature for 10 min. Transfer the upper N-hexane; take 50µl Lower phase for the analysis. (Dilution times of the sample: 1:2)
- **Honey, Tissue, Prepared intestine, Liver, Egg**: Weight 1 g homogeneous samples into the 50 ml centrifuge tube, pipette 4 ml deionized water, 0.15 ml 1M HCl, and 100 µl Derivatization reagent, oscillate 5 min. Incubate in 37°C water bath overnight or in 50°C for 3 hours. 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. Wipe out 2.5 ml supernatant to another centrifuge tube and blow dry at 50 to 60°C with nitrogen or air. Add 1 ml N-hexane to dissolve the dried residue, and then add 1 ml redissolving solution, oscillate for 30 sec, centrifuge at 4000 rpm at room temperature for 10 min. Remove the upper N-hexane; take 50 µl Lower phase for the analysis. (Dilution times of the sample: 1:2)
- **Cooked Food**: Weight 1 g homogeneous samples into the 50 ml centrifuge tube, pipette 4.5 ml methanol and 0.5 ml deionized water, oscillate 2 min, centrifuge at 4000 rpm at room temperature for 5 min, discard all the liquid. Add 5 ml acetonitrile and 5 ml N-hexane. oscillate for 2 min, centrifuge at 4000 rpm at room temperature for 5 min, and discard all the liquid. Pipette 4 ml deionized water, 0.5 ml 1 M HCl and 100µl derivatization reagent into the precipitation, oscillate 5min. Incubate in 37°C water bath overnight or in 50°C for 3 hours. 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. Wipe out 2.5 ml supernatant to another centrifuge tube and blow dry at 50 to 60°C with nitrogen or air. Add 1 ml N-hexane to dissolve the dried residue, and then add 1 ml redissolving solution, oscillate for 30 sec, centrifuge at 4000 rpm at room temperature for 10 min. Remove the upper N-hexane; take 50 µl Lower phase for the analysis. (Dilution times of the sample: 1:2)

VIII. 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 VII.
2. Add 50 µl **diluted standards** or **samples** into marked well. Add 50 µl **Enzyme Conjugate** into each well, then add 50 µl **antibody working solution** into each well.
3. Cover with the adhesive Membrane ,oscillate the plate for 5 sec, and incubate in dark 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.
6. Add 50 µl **Stop Solution** to each well and oscillate gently to stop the reaction.
7. Read result at 450 nm within 10 minutes.

IX. 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 Sal of samples.

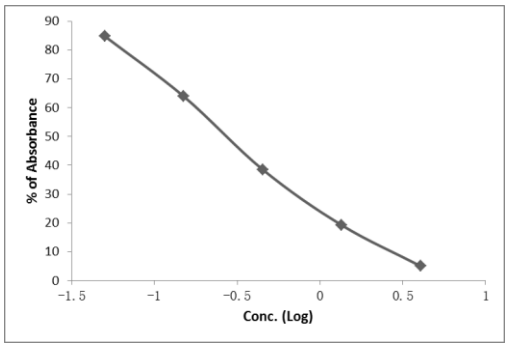

Figure: Typical Standard Curve: These standard curves are for demonstration only. A standard curve must be run with each assay.

FOR RESEARCH USE ONLY! Not to be used on humans.

X. Related Products:

- Gentamicin ELISA Kit (Cat. No. K4206-100)
- Kanamycin ELISA Kit (Cat. No. K4210-100)
- Streptomycin ELISA Kit (Cat. No. K4272-100)
- Fluoroquinolones ELISA Kit (Cat. No. K4205-100)

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