

Melamine ELISA Kit

rev 07/18

(Catalog # E4274-100, 100 assays, Store at 4°C)

I. Introduction:

Melamine is an organic base and a trimer of cyanamide, with a 1,3,5-triazine skeleton. Melamine is harmful if swallowed, inhaled or absorbed through the skin. Chronic exposure may cause cancer or reproductive damage or eye, skin and respiratory irritant. When melamine and cyanuric acid are absorbed into the bloodstream, they concentrate and interact in the urine-filled renal tubules, then crystallize and form large numbers of round, yellow crystals, which in turn block and damage the renal cells that line the tubes, causing the kidneys to malfunction. BioVision's Melamine ELISA kit is a competitive ELISA assay for the quantitative measurement of Melamine in milk powder, milk, tissue, feed, egg, serum. The density of color is proportional to the amount of melamine captured from the samples.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of Melamine.

Detection Range: 2- 162 ppb (ng/ml)

Sensitivity: < 1 ppb

Detection limit: 2 ppb for milk powder and milk, 4 ppb for tissues, 200 ppb for feed, 40 ppb for egg, 8 ppb for serum

III. Sample Type:

Milk powder, milk, tissue, feed, egg, serum

IV. Kit Contents:

Components	E4274-100	Part No.
Micro ELISA Plate	8 X 12 strips	E4274-100-1
Standards (S1 – S6)	1.0 ml X 6	E4274-100-2-x
High standard (1000 ppb)	1.0 ml	E4274-100-3
Antibody working solution	5.5 ml	E4274-100-4
Enzyme conjugate	5.5 ml	E4274-100-5
Substrate A solution	6 ml	E4274-100-6
Substrate B solution	6 ml	E4274-100-7
Stop Solution	6 ml	E4274-100-8
Concentrated Wash Solution (20X)	40 ml	E4274-100-9
Concentrated Redissolving solution (2X)	50 ml	E4274-100-10
Adhesive Membrane	1	E4274-100-11

V. User Supplied Reagents and Equipment:

- Reagents: 1M HCl, 0.1M NaOH, acetonitrile-0.1 M NaOH solution, 1M NaOH, N-hexane
- Microplate reader capable of measuring absorbance at 450 nm
- Precision pipettes with disposable tips
- Distilled or deionized water
- 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. Reagent 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	2	6	18	54	162

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 °C 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:**

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/ Milk Powder:** Take 2ml milk sample or 2g milk powder into the centrifuge tube. Add 8ml acetonitrile-0.1 M NaOH solution, oscillate for 4min fully, centrifuge at room temperature at 4000 r/min for 10 min, take 4ml supernatant into glass tube and blow dry at 50 -60 °C

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with nitrogen or air. Dissolve dried materials with 1ml N-hexane, then add 1ml redissolving solution, mix for 30s, centrifuge and remove the upper N-hexane. Take 50µl lower water phase for the analysis. (Dilution factor: 1)

- **Tissue:** Weight 2 g homogeneous samples into the 50ml centrifuge tube. Add 8ml acetonitrile-0.1 M NaOH solution, oscillate for 2min fully, centrifuge at room temperature at 4000 r/min for 10 min, take 2ml supernatant into glass tube and blow dry at 50 -60 °C with nitrogen or air. Dissolve dried materials with 1ml N-hexane, then add 1ml redissolving solution, mix for 30s, centrifuge and remove the upper N-hexane. Take 50µl lower water phase for the analysis. (Dilution factor: 2)
- **Feed:** Weight 2.0 g crushed feed samples; add 2 ml 1M HCl and 16ml deionized water, mix fully. Swirl 1min, put on the oscillator 2min. Centrifugal at room temperature at 4000r / min for 15min, take out 10ml supernatant and use 1M NaOH to adjust PH value to 6-8. (Note: Due to different feed samples, the addition of 1M NaOH in Vary, depending on the circumstances adjustment range is typically added between 0.5 - 1ml) Centrifugal at room temperature at 4000r / min for 15min, take out supernatant (if supernatant is still cloudy, can available Increase the speed or filtered with a filter paper). Take out supernatant and use redissolving solution to dilute 10 times. (take 100ul supernatant and add 900ul redissolving solution, mix evenly) Take 50µl for the analysis. (Dilution Factor: 100)
- **Egg:** Mix the sample slowly with homogenizer (egg white, egg yolk or egg). Weight 2.0 g homogeneous samples, add 8 ml acetonitrile-0.1M NaOH solution, and oscillate for 2 min fully. Centrifuge at room temperature at 4000 r/min for 10 min, take 1ml supernatant into glass tube and blow dry at 50 -60 °C with nitrogen or air. Dissolve dried materials with 1ml N-hexane, then add 1ml redissolving solution, mix for 30s, centrifuge and remove the upper N-hexane. Take 50 µl lower water phase and add 150ul redissolving solution, mix for 30s. Take 50µl for the analysis. (Dilution Factor: 40)
- **Serum:** Take 0.5ml serum sample into the 50ml centrifuge tube. Add 2ml acetonitrile-0.1 M NaOH solution, oscillate for 2min fully, centrifuge at room temperature at 4000 r/min for 10 min, take 1ml supernatant into glass tube and blow dry at 50 -60 °C with nitrogen or air. Dissolve the dried materials with 1ml N-hexane, then add 1ml redissolving solution, mix for 30s, centrifuge and remove the upper N-hexane. Take 50µl for the analysis. (Dilution Factor: 4)

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.
3. Oscillate the plate for 5 sec, cover the well and incubate in dark for 30 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 (%) = $A/A_0 \times 100\%$

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 the samples.

X. 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. K4210-100)
- Streptomycin ELISA Kit (Cat. No. E4272-100)
- Tetracyclines ELISA Kit (Cat. No. E4273-100)

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