Melamine ELISA Kit

Catalog Number. CSB-E12003f

For the quantitative determination of melamine concentrations in milk powder, liquid milk, fish and shrimp, chicken, feedstuff.

This package insert must be read in its entirety before using this product.

In order to obtain higher efficiency service, please ready to supply the lot number of the kit to us (found on the outside of the box).

PRINCIPLE OF THE ASSAY

This assay employs the competitive inhibition enzyme immunoassay technique. The microtiter plate provided in this kit has been pre-coated with melamine antigen. Standards or samples are added to the appropriate microtiter plate wells with an antibody specific for melamine and Horseradish Peroxidase (HRP) conjugated secondary antibody. The competitive inhibition reaction is launched between with pre-coated melamine and melamine in samples. A substrate solution is added to the wells and the color develops in opposite to the amount of melamine in the sample. The color development is stopped and the intensity of the color is measured.

DETECTION RANGE

2 ppb-162 ppb.

SENSITIVITY

The minimum detectable dose of melamine is typically less than 2 ppb.
The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest concentration of melamine that could be differentiated from zero. It was determined the mean O.D value of 20 replicates of the zero standard added by their three standard deviations.

Detection limit

Tissue	4ppb
Milk powder	40ppb
Liquid milk	32ppb
Feedstuff	200ppb

Cross-reaction rate

Melamine	100%
Tricyanicacid	48%
3,5-Triazine-2,4-diamine	＜1%

Recovery rate

Tissue	90% ±10%
Milk powder	95% ±10%
Liquid milk	90% ±10%
Feedstuff	90% ±10%

PRECISION

Intra-assay Precision (Precision within an assay): CV%<10%
Three samples of known concentration were tested twenty times on one plate to assess.

Inter-assay Precision (Precision between assays): CV%<10%
Three samples of known concentration were tested in twenty assays to assess.

LIMITATIONS OF THE PROCEDURE

- **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples and repeat the assay.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

MATERIALS PROVIDED

Reagents	Quantity
Assay plate	1(96 wells)
Standard	6 x 1 ml
Antibody	1 x 7 ml
HRP-conjugate	1 x 12 ml
Substrate A	1 x 7 ml
Substrate B	1 x 7 ml
Stop Solution	1 x 7 ml
Wash Buffer (20 x concentrate)	1 x 40 ml
Redissolving Solution (2×concentrate)	1 x 50 ml
Adhesive Strip (For 96 wells)	4
Instruction manual	1

STANDARD CONCENTRATION

Standard	S0	S1	S2	S3	S4	S5
Concentration (ppb)	0	2	6	18	54	162

STORAGE

Unopened kit	Store at 2 - 8°C. Do not use the kit beyond the expiration date.
Opened kit	May be stored for up to one month at 2 - 8° C.

***Provided this is within the expiration date of the kit.**

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- An incubator which can provide stable incubation conditions up to 37°C±0.5°C.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- Absorbent paper for blotting the microtiter plate.
- 100ml、500ml and 1000ml graduated cylinders.
- Deionized or distilled water.
- Pipettes and pipette tips.
- Test tubes for dilution.
- Acetonitrile, NaOH, HCl

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

REAGENT PREPARATION

Note:

- **Kindly use graduated containers to prepare the reagent. Please don't prepare the reagent directly in the Diluent vials provided in the kit.**
- Bring all reagents to room temperature (18-25°C) before use for 30min.
- Distilled water is recommended to be used to make the preparation for reagents. Contaminated water or container for reagent preparation will influence the detection result.

1. **0.1M NaOH Solution**: dissolve 0.4 g NaOH in deionized water to 100ml.
2. **1 M HCl**: dissolve 8.6 ml HCl (36%) in deionized water to 100 ml.
3. **1 M NaOH Solution**: dissolve 4 g NaOH in deionized water to 100 ml.
4. **Acetonitrile-0.1M NaOH**: Take 84ml acetonitrile and mix with 16ml 0.1M NaOH Solution.

5. **Redissolving Solution:** The 2 concentrated redissolving solution is diluted with deionized water at 1:1 (1ml concentrated redissolving solution + 1ml deionized water).

6. **Wash Buffer(1x)-** If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 40 ml of Wash Buffer Concentrate (20 x) into deionized or distilled water to prepare 800 ml of Wash Buffer (1 x).

SAMPLE COLLECTION AND STORAGE

A: Shrimp, fish and chicken(Fold of dilution of the samples: 2)

(1) Weigh 2.0±0.05 g of the homogenized sample, put into 50ml centrifugal tube, add 8ml Acetonitrile/0.1M NaOH mixed solution shake properly for 5min. Centrifuge at more than 4000 r/min for 10 min at room temperature.
(2) Transfer 2ml supernatant into a new centrifugal tube, evaporated to dryness at 56°C by nitrogen or rotary evaporator.
(3) Add 1ml of the N-hexane to redissolve drying residue. Then add 1ml diluted redissolving solution and shake strongly for 30s. Centrifuge at more than 4000 r/min at room temperature for 5 min.
(4) Remove the upper layer. Take 50μl for further analysis.

B: Liquid milk (Fold of dilution of the samples: 16)

(1) Take 600μl milk into 2ml centrifugal tube. Add 1ml Acetonitrile. Mix well.
(2) Take 100μl supernatant into 900μl of diluted redissolving solution. Mix well.
(3) Take 50μl for further analysis.

C: Milk powder(Fold of dilution of the samples: 20)

(1) Weigh 2.0±0.05 g of sample, put into 50ml centrifugal tube, add 4ml methanol shake properly for 5min. Centrifuge at more than 4000 r/min for 10 min at room temperature.
(2) Take 100μl supernatant into 900μl of diluted redissolving solution. Mix well.
(3) Take 50μl for further analysis.

D: Feedstuff (Fold of dilution of the samples: 100)

(1) Weigh 2.0±0.05 g of the homogenized sample, put into 50ml centrifugal tube, add 2ml 1M HCl and 16ml ddH_2O shake properly for 5min. Centrifuge at more than 4000 r/min for 15 min at room temperature.

(2) Transfer 10 ml supernatant into a new centrifugal tube, adjust to pH6-8 with 1M NaOH(Normally need about 0.5-1ml NaOH). Centrifuge at more than 4000 r/min for 15 min at room temperature.

(3) Take 100μl supernatant into 900μl of diluted redissolving solution. Mix well.

(4) Take 50μl for further analysis.

Note:

1. CUSABIO is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
2. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
3. Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. Centrifuge the sample again after thawing before the assay. It is recommended that all samples and standards be assayed in duplicate.

1. Prepare all reagents, and samples as directed in the previous sections.
2. Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the ziploc, store unused wells at 4°C.
3. Add 50µl of **standard and sample** per well. Add 50µl of **Antibody** to each well. Mix well. Cover with the adhesive strip provided. Incubate for 30 minutes at room temperature (25°C). **Protect from light.**
4. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Buffer (250µl) using a squirt bottle, multi-channel pipette, manifold dispenser, or autowasher, and let it stand for 15-30 seconds, complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating ordecanting. Invert the plate and blot it against clean paper towels.
5. Add 100µl of **HRP-conjugate** to each well. Cover the microtiter plate with a new adhesive strip. Incubate for 30 minutes at room temperature (25°C). **Protect from light.**
6. Repeat the aspiration/wash process for five times as in step 4.
7. Add 50µl of **Substrate A** and 50µl of **Substrate B** to each well. Incubate for 15 minutes at room temperature (25°C). **Protect from light.**
8. Add 50µl of **Stop Solution** to each well, gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well within 5 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 630 nm. Subtract readings at 630 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

Note:

1. The final experimental results will be closely related to validity of the products, operation skills of the end users and the experimental environments.
2. Samples or reagents addition: Please carefully add samples to wells and mix gently to avoid foaming. Do not touch the well wall as possible. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. This will ensure equal elapsed time for each pipetting step, without interruption. Duplication of all standards and specimens, although not required, is recommended. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
3. Incubation: To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added to the well strips, DO NOT let the strips DRY at any time during the assay. Incubation time and temperature must be observed.
4. Washing: The wash procedure is critical. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting and remove any drop of water and fingerprint on the bottom of the plate. Insufficient washing will result in poor precision and falsely elevated absorbance reading. When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
5. Stop Solution should be added to the plate in the same order as the TMB Substrate. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the TMB Substrate.

ASSAY PROCEDURE SUMMARY

1. Prepare reagents, samples as instructed.
2. Add 50μl standard or sample to each well.
3. Add 50μl of Antibody to each well.
4. Incubate 30 minutes at room temperature. Protect from light.
5. Aspirate and wash 5 times.
6. Add 100μl HRP-conjugate to each well.
7. Incubate 30 minutes at room temperature. Protect from light.
8. Aspirate and wash 5 times.
9. Add 50μl Substrate A and 50μl Substrate B to each well. Incubate 15 minutes at room temperature. **Protect from light.**
10. Add 50μl Stop Solution to each well. Read at 450nm within 5 minutes.

CALCULATION OF RESULTS

Using the professional soft "Curve Expert 1.3" to make a standard curve is recommended, which can be downloaded from our web.

(1) The mean values of the 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.

$$\text{Absorbency value}\ (\%) = \frac{B}{B_0} \times 100\%$$

B ——absorbance standard (or sample)
B0 ——absorbance zero standard

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

The melamine 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 the actual concentration of sample is obtained.