AccuEZ^{TM} Melamine Fast Detection ELISA Kit

User Guide

(Catalog No. AAF-21101)

Enzyme-Linked Immunosorbent Assay Kit for Detection of Melamine in Dairy Products

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General Description

Melamine is a trimer of cyanamide with the chemical formula $C_3H_6N_6$, and the IUPAC name 1,3,5-triazine-2,4,6-triamine. It is widely used in manufacturing of plastics, dyes, fertilizers, and textile. Like cyanamide, it is 66% nitrogen by mass, which leads to the practice in various countries of adding "melamine scrap" to animal feed, pet food, or milk in order to give the appearance of increased protein content. This practice can potentially contaminate animal products intended for human consumption like dairy products. Animal and human consumption of melamine could lead to kidney stones and renal failure, causing severe consequences including possible death.

AccuEZ™ melamine fast detection ELISA kit allows for **fast** (entire procedure in 30 minutes), **simple** (direct analysis for dairy products), **sensitive** and **reliable** detection of melamine contamination in dairy products such as milk, milk powder and yogurt. If necessary, samples requiring regulatory action can be confirmed by HPLC, GC/MS, or other conventional methods.

Intended Use

AccuEZ™ Melamine Fast Detection ELISA Kit is a competitive ELISA for the quantitative and qualitative analysis of melamine in dairy products including milk, milk powder and yogurt. The limit of detection (LOD) of melamine for dairy products is 1 ppb (1 ng/ml).

Safety Instructions

To receive complete safety information on this product, contact AccuAffinity, Inc. and request Material Safety Data Sheets. Stop solution is 1N sulfuric acid. Handle with care.

Assay Principle

AccuEZ™ Melamine kit is a competitive enzyme-labeled immunoassay. Each well of the 96-well plate has been pre-coated with mouse monoclonal anti-melamine antibody. During assay, melamine standard solution or samples are added to test wells, followed by adding horse radish peroxidase (HRP)-melamine conjugate, which will compete with melamine in standard/sample for binding to antibody during 20-minute incubation. After washing the plate, a clear HRP substrate is added to the wells leading to a colored product, and optical density is inversely related to melamine concentration in the samples. The accurate concentration of melamine can then be determined by interpolation using the standard curve constructed in the same run.

Reagents and Materials Provided

The reagents included in the kit are sufficient for performing 96 measurements (including standards and samples). Reagents and materials in each kit include:

a) 1 microtiter plate containing 12 test strips of 8 wells sealed in an aluminized pouch with dessicant.
b) 6 vials each containing 0.5 mL of melamine standard with 0, 2, 10, 50, 250 and 1250 ng/mL of melamine respectively.
c) 1 bottle containing 10 mL melamine-HRP conjugate (1×).
d) 1 bottle containing 15 mL microtiter plate wash solution (20×).
e) 1 bottle containing 11 mL substrate (1×).
f) 1 bottle containing 11 mL stop solution (1×).
g) 2 microtiter plate sealers.
h) 1 booklet of instruction

Materials Required but not Provided

a) Microplate reader with 450 nm filter.
b) Pipet capable of dispensing 20-200 µl

(All reagents needed for performing the test are included in the kit.)

Assay Procedure

- Equilibrate kit components at room temperature (20-25^{o}C) for at least 30 min prior to running the test, and thoroughly mix all liquid components before use.
- Use test strips as needed on the frame, and store unused strips in the re-closable bag at 2-8^{o}C.
- Number standards and samples according to positions on microtiter plate. All standards and samples need duplicate measurement for accuracy.

Dairy product samples do not need any pretreatment. When measuring milk powder samples, dissolve 1 g milk powder into 6 mL H_2O to reconstitute to its original concentration.

1. Sample addition: Dispense **20 µl standard or sample** into each well with pipet.
2. Enzyme conjugate addition: Add **80 µl melamine-HRP conjugate** into each well.
3. Incubation: Gently hand-shake plate to thoroughly mix for about 1 minute, seal the plate with a plate sealer, and incubate at room temperature (20-25^{o}C) for **20 min** without shaking.
4. Plating washing: Dilute appropriate amount of 20× microtiter plate wash solution to 1×, decant reaction mixture from wells, and add **200 µl 1× wash solution** and dump. Repeat wash step for a total of **4 washes**. Following the last wash, tap the inverted wells onto absorbent paper to completely remove wash solution.
5. Color reaction: Dispense **100 µl substrate solution** into each well. Incubate at room temperature (20-25^{o}C) while avoiding light for **10 min**.
6. Add **100 µl stop solution** to each well and mix by shaking gently. Measure absorbance of the wells at 450 nm (OD_{450} value) with microplate reader.

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Calculating Results

Two approaches can be used to obtain melamine concentration results from the assay. Semi-quantitative results can be obtained with the first approach while quantitative results can be calculated with the second. Please note the negative correlation between absorbance reading (OD_{450}) and melamine concentration in the sample.

1. Semi-quantitative detection of melamine

A simple comparison of average sample absorbance to absorption of standards will give the range of melamine concentration (ng/mL or ppb) in the samples. For example, Sample 1 has an average absorbance of 0.6, and Sample 2 of 1.2, and OD_{450} of standard solutions are as follows: 2.300 for 0 ng/mL; 2.000 for 2 ng/mL; 1.650 for 10 ng/mL; 0.950 for 50 ng/mL; 0.400 for 250 ng/mL; 0.165 for 1250 ng/mL. It is immediately known that melamine concentration of Sample 1 is between 50 ng/mL and 250 ng/mL, while Sample 2 contains 10-50 ng/mL melamine.

2. Quantitative calculation of melamine concentration

a) Calculate B/B_0
Dividing average absorbance of each standard and sample (B) by absorbance of first standard (the standard with 0 ng/mL melamine concentration, B_0) to obtain percentage absorbance.
percentage absorbance (%) = $B/B_0 \times 100\%$
B — average absorbance of a standard or sample
B_0 — average absorbance of 0 ng/mL standard

b) A standard curve is obtained by graphing the percentage absorbance of standards (Y axis) versus their corresponding concentration (X axis) on semi-log graph paper (which should be a linear relationship), and sample concentration can be read from this standard curve. Alternatively, melamine concentration in the samples can be calculated with regression equation correlating percentage absorbance to melamine concentration. Graphing software can also be used for quick analyses of large number of samples.

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Performance Data

Range of Standard Curve: 0-1250 ng/mL
Assay Quantitative Range: 2-1250 ng/mL
Assay Time: 30 min
Limit of Detection (LOD):

Milk	1 ppb
Milk powder (as milk)	1 ppb
Yogurt	1 ppb

Recovery:

Milk, Milk powder, Yogurt	85-115%

Specificity:

Melamine	100%

Sensitivity (defined as the average of absorbance from 6 zero-standards minus 3 times of standard deviation):
1 ng/mL (1 ppb)

Precision:

Intra-assay CV

For 1000 ng/mL sample:	5.8%
For 100 ng/mL sample:	5.2%
For 10 ng/mL sample:	12.7%

Inter-assay CV

For 1000 ng/mL sample:	6.1%
For 100 ng/mL sample:	7.2%
For 10 ng/mL sample:	17.9%

EC50: 30 ng/mL (7.1% CV)

Spiking Recovery:
Average recovery of melamine spiked into 6 different milk samples and 5 milk powder samples

For 500 ng/mL (500 ppb) sample concentration:	93.6%
For 50 ng/mL (50 ppb) sample concentration:	95.5%
For 25 ppm sample concentration:	92.2%
For 10 ppm sample concentration:	87.7%

For 5 ppm sample concentration: 94.0%

Accuracy:

For 1000 ng/mL sample: average measured result 1060.9 ng/mL (106.1%)
For 100 ng/mL sample: average measured result 102.1 ng/mL (102.1%)
For 10 ng/mL sample: average measured result 10.2 ng/mL (101.5%)

Precautions

1. Assay kit should be stored at 2-8^{o}C and avoid freezing conditions; unused test strips should be sealed in reclosable bag; colorless substrate is sensitive to light so prolonged exposure to light needs to be avoided.
2. Reagents should be brought to room temperature (20-25^{o}C) prior to use. A room temperature of lower than 20^{o}C or failure to equilibrate reagents or samples to room temperature could result in low OD readings for all samples. All reagents should be put back into 2-8^{o}C storage immediately after use.
3. Adhere to assay protocol on reaction temperature and time, and use pipet to add components whenever possible. Results are solely based on OD450 readings from plate/strip reader.
4. Reagents need to be thoroughly mixed to improve reproducibility.
5. During all incubation steps, avoid light and seal plate with sealer.
6. If wells are dried out during plate wash steps, linearity of standard curve will be negatively affected and reproducibility will be poor. Therefore, substrate addition should be carried out immediately after tapping the plate dry (following the last wash).
7. The stop solution is 1N sulfuric acid. Avoid contact with skin or clothing. Immediately clean up any spills and wash area with copious amounts of water. If contact should occur, immediately flush with copious amounts of water.
8. Do not use reagents beyond expiration date. Dilution or adulteration of reagents or samples not called for in the procedure may result in adverse changes in sensitivity and OD reading. Do not substitute reagents from kits with different lot numbers.
9. Obvious color in substrate suggests expiration and it should be discarded. When absorbance of zero-standard is lower than 0.8, the reagents may have expired.

Storage and Expiration Date

Storage: All components of the kit should be stored at 2-8^{o}C.
Expiration Date: This kit expires 12 months after manufacturing date.

Technical Assistance

For ordering or technical assistance regarding this kit, or for additional information about AccuAffinity products, please email support@accuaffinity.com or call (408) 368-1364.

General Limited Warranty

AccuAffinity, Inc. warrants its manufactured products against defects in materials and workmanship when used in accordance with the applicable instructions for a period not to extend beyond a product's printed expiration date. AccuAffinity makes no other warranty, expressed or implied. There is no warranty of merchantability or fitness for a particular purpose. The warranty provided herein and the data, specifications and descriptions of AccuAffinity products appearing in published catalogues and product literature may not be altered except by express written agreement signed by an officer of AccuAffinity. Representations, oral or written, which are inconsistent with this warranty or such publications are not authorized and, if given, should not be relied upon.

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