Melamine Fast ELISA (Catalog: TBS21104)

One-step-One-Hour ELISA For determination of melamine concentrations in serum, urine, tissue, and animal feed

Description

Melamine is a trimmer of cyanamide with the chemical formula C3H6N6, and the IUPAC name 1, 3, 5-triazine-2, 4, 6-triamine. It is widely used in manufacturing plastics, dyes, fertilizers, and textiles. 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.

Tribioscience's Melamine Fast ELISA kit allows for fast (entire procedure in 30 minutes), simple (direct analysis for dairy products; only homogenization and water extraction are needed for animal feed or pet food), sensitive and reliable detection of melamine contamination in animal feed, pet food and 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

Tribioscience Melamine Fast ELISA Kit is a competitive ELISA for the quantitative analysis of melamine in animal feed, pet food and dairy products including milk, milk powder and yogurt.

The limit of detection (LOD) of melamine for animal feed or pet food is 200 ppb, and LOD for dairy products is 1 ppb (1 ng/ml).

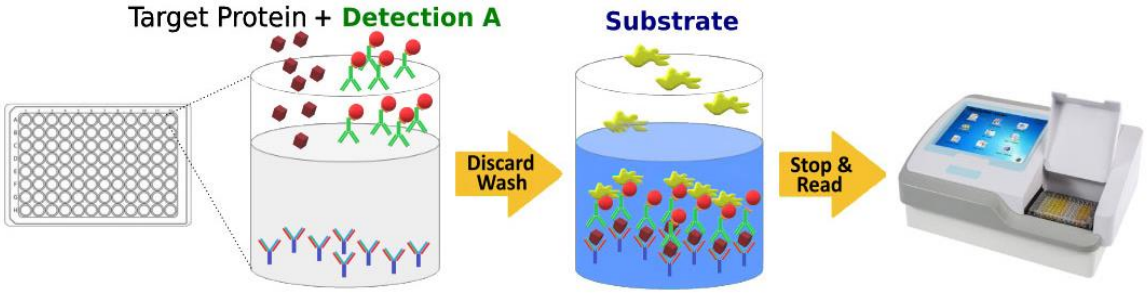
Safety Instructions

To receive complete safety information on this product, contact Tribioscience, Inc. and request Material Safety Data Sheets (MSDS).

Assay Principle

The Melamine Fast ELISA Kit is a competitive enzyme-labeled immunoassay (Fig. 1). The 96-well plate has been pre-coated with an anti-melamine antibody. During the assay, melamine standard or samples are added to test wells, followed by adding horse radish peroxidase (HRP)-melamine conjugate, which will compete with melamine in standard or sample for binding to antibody during the 30-minute incubation. After plate wash, an ultra-sensitive TMB substrate is added to the wells leading to a colored product only in the presence of HRP, and optical density is inversely related to melamine concentrations in the samples. The accurate concentration of melamine can then be determined by using the standard curve constructed in the same run.

Fig. 1. Simple Procedures



KIT CONTENT AND STORAGE CONDITIONS

PART	PART#	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED
Pre coated Microplate	TBS21104A	96 well microplate (12 strips of 8 wells) coated with an antibody specific for melamine.	Return unused wells to the foil pouch. Reseal along the entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.
Melamine Standard	TBS21104B	60 µL of melamine (8.1 µg/mL).	Aliquot and store at -20 °C for up to 1 month in a manual defrost the freezer. Avoid repeated freeze-thaw cycles.
Detection A	TBS21104C	200 µL of HRP- melamine conjugate (100x)	May be stored for up to 4 months at 2-8 °C.
Assay Diluent	TBS21104D	25 mL of a buffered protein base with preservatives.	
Wash Buffer	TBS3000W	12 mL of concentrated solution (10x).	
TMB Substrate	TBS3000T	10 mL of ultra-sensitive TMB substrate.	
Stop Solution	TBS3000S	6 mL of 1 N sulfuric acid.	

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

Sample preparation
Dariy product sample:
Dairy product samples do not need any pretreatment. When measuring milk powder samples, dissolve 1 g milk powder into 6 mL H2O to reconstitute to its original concentration.

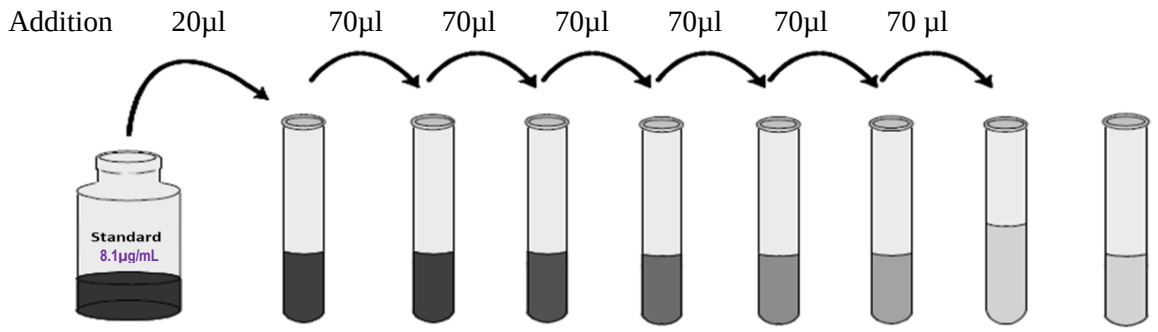
Animal feed sample:
1. Animal feed or pet food first needs to be homogenized into fine powder (or in the case of moist pet food, into pudding-like paste).
2. Weigh 1 g of homogenized sample, add 10 mL H2O, and mix thoroughly by vigorous vortex. Incubate at room temperature for at least 15 min.
3. Centrifuge at 4000 rpm for 3 min at room temperature.
4. Take 10 µl of cleared supernatant and mixed with 190 µl sample dilution buffer. Final concentration corresponds to a 200-fold dilution from original sample.

Assay Procedure
Equilibrate kit components at room temperature (20-25 °C) for at least 30 min prior to running the test, and thoroughly mix all liquid components before use.

Wash Buffer: Add 12 mL of Wash Buffer Concentrate(10x) to 108 mL of deionized distilled water to prepare 120 mL of Wash Buffer.
Detection A (HRP-Melamine Conjugate): Prepare HRP working solution by diluting 1 part of HRP conjugate (100x) with 99 parts of Assay Diluent (1x) to HRP working solution (1x). For 1 plate, add 60 µL HRP-conjugate (100x) to 6 mL Assay Diluent.

Melamine Standard Preparation:
1. Label test tubes as #1 through #8.
2. Pipet 180 µL of 1x Assay Diluent into tube #1, and 14 µL into tubes #2 to #8 as diagram below (Fig.2).
3. Add 20 µL of Melamine Standard stock solution (8.1µg/mL) to tube #1(810ng/mL) and mix.
4. Make 3x serial dilutions of the standard using the Tube#1(810 ng/mL standard solution) from Tube #2 through #7 with sequential transfer of 70 µL to the next concentration. Mix each tube thoroughly before the next transfer. The standard concentration in tube 1 through 7 will be 810, 270, 90, 30, 10, 3.3, and 1.1 ng/mL. Tube# 8 is Standard 0.

Fig.2 Diagram for melamine standard preparation



	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std 8
Assay Buffer (µL)	180	140	140	140	140	140	140	140
Addition	Stock	Std1	Std2	Std3	Std4	Std5	Std6	
Addition Vol. (µL)	20	70	70	70	70	70	70	
Final Conc (ng/ml)	810	270	90	30	10	3.3	1.1	0

Assay Procedures:

1. Add 50 µL of standard, sample, or control per well.
2. Add 50 µL of **Detection A** to the above standard and sample of each well, thoroughly mix. Cover with the adhesive sealer. Incubate at **RT for 30min**.
3. Aspirate each well, and wash for 3 times by filling each well with 200 µL Wash Buffer. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
4. Add 100 µL of **TMB Substrate** to each well. Incubate **at RT for 10-20min** (*Protect from light*). The color becomes blue.
5. Add 50 µL of **Stop Solution** to each well. The color in the well should change from blue to yellow (gently tap the plate to ensure thorough mixing).
6. Determine the optical density of each well within 20 minutes, using a microplate reader at 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 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.

Quantitative Calculation of Melamine Concentration

A: Calculate B/B0: Dividing average absorbance of each standard and sample (B) by absorbance of the control of 0 ng/mL melamine concentration, B0) to obtain percentage absorbance.

Percentage absorbance (%) = 100%*(B/B_0).

B: Average absorbance of a standard or sample.
B_0: Average absorbance of 0 ng/mL control.

B: A standard curve is obtained by graphing the percentage absorbance of standards (Y axis) versus their corresponding concentration (X axis), and melamine concentration of 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 samples.

Performance Data

Range of Standard Curve: 0-810 ng/mL
Assay Quantitative Range: 5-1280 ng/mL
Assay Time: 50 min.

Limit of Detection (LOD):

Milk:	1 ppb.
Milk powder (as milk):	1 ppb.
Yogurt:	1 ppb
Animal feed/pet food:	200 ppb

Recovery:
Milk, Milk powder, Yogurt: 85-115%
Animal feed/pet food: 87-98%

Specificity:
Melamine 100%

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

Precision:

Intra-assay CV	<10%
Inter-assay CV	<15%

Precautions
1. Assay kit should be stored at 2-8℃ and avoid freezing conditions; unused test strips should be sealed in re-closable 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℃) prior to use. A room temperature of lower than 20℃ 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℃ storage immediately after use.
3. Do not use reagents beyond expiration date.

Technical Assistance
For ordering or technical assistance regarding this kit, or for additional information about Tribioscience products, please email: support@tribioscience.com or call (408) 498-0197, or 833-697-8998 (Toll Free).

Relative Product
Melamine rapid detection test strip (TBS11102)
Clenbuterol Rapid Test for Urine Samples (TBS11111)
Clenbuterol Rapid Test for Tissue Samples (TBS11112)
Chloramphenicol test strip (TBS11121)
Ractopamine rapid detection test strip (TBS11131)
Salbutamol rapid detection test strip (TBS11141)
Shiga Toxin (STX) Rapid Test Strip (TBS11151)
Vomitoxin / Deoxynivalenol (DON) Test Strip (TBS11156)
Ochratoxin A test strip (TBS11161)
Aflatoxin B1 test strip (TBS11166)
Zearalenone (ZEA) test strip (TBS11171)
Chloramphenicol Fast ELISA (TBS21121)
Clenbuterol Fast ELISA (TBS21111)
Total Aflatoxin Fast ELISA(TBS21131)
Ochratoxin-A Fast ELISA(TBS21133)
Salbutamol ELISA (TBS21141)

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