

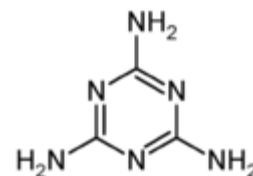


# AgraQuant<sup>®</sup> Melamine Sensitive Assay

Order #: COKAQ9400

## Intended Use

The AgraQuant<sup>®</sup> Melamine Sensitive Assay is a direct competitive enzyme-linked immunosorbent assay (ELISA) that determines a quantitative level of melamine and is intended for use in milk, milk powder and other dairy products.



Melamine

## Melamine

Melamine is an organic base with the chemical formula of  $C_3H_6N_6$  and the IUPAC name of 1,3,5-triazine-2,4,6-triamine. It is often combined with formaldehyde to produce melamine resin, a synthetic polymer which is fire resistant and heat tolerant.

Melamine became a topic of much discussion in early 2007, when veterinary scientists determined it to be the cause of hundreds of pet deaths, because of pet food contamination. Prior to these reports, melamine had been regarded as non-toxic or minimally toxic. However, because of the unexplained presence of melamine in wheat gluten added to dog and cat foods, it is the most likely cause. Pet owners reported symptoms that are commonly associated with renal failure, which could be explained by the ammonia that may result from the digestion of the melamine.

It had been reported in September 2008 that melamine was found in milk and infant formula produced by several Chinese dairy companies, which led to kidney stones and other renal failure among young children in China. By 22 September, more than 50,000 people had become ill, with 4 infant deaths and more than 12,800 hospitalizations.

Melamine has been added into raw milk to give appearance of higher protein content than the true value. This is because of melamine's high nitrogen content. The AgraQuant<sup>®</sup> Melamine Sensitive Assay is able to detect 0.1ppm melamine in milk and 0.5ppm melamine in milk powder. The quantitation ranges of test kit for milk and milk powder are 0.1-5.0ppm and 0.5-25ppm, respectively.

## Assay Principles

The AgraQuant<sup>®</sup> Melamine Sensitive Assay is a direct competitive enzyme-linked immunosorbent assay (ELISA) designed for dairy industry. The sample and enzyme-conjugated melamine are pipetted into the antibody-coated microwell. Melamine from the samples and control standards are allowed to compete with enzyme-conjugated melamine for the antibody binding sites during the first incubation period. The microwells are then washed with de-ionized water. After the washing step, a substrate is added to the wells and blue color develops. The intensity of the color is inversely proportional to the concentration of melamine in the sample or standard. A stop solution is then added which changes the color from blue to yellow. The microwells are measured optically using a microwell reader with an absorbance filter of 450nm. The optical densities (OD) of the samples are compared to the OD's of the standards and an interpretative result is determined.

## Precautions

1. Store reagents at 2-8°C (35-46°F) when not in use, and do not use the kit beyond its expiration date.
2. Adhere to incubation times stated in the procedure. Use of incubation times other than those specified may give inaccurate results.

3. Each reagent is optimized for use in the melamine kit. Do not combine reagents from other melamine kits with different lot numbers.
4. Melamine is a potential toxic chemical and should be treated with care.
5. The Stop Solution contains acid. Avoid contact with skin or eyes. If exposed, flush with water.
6. Wear protective gloves and safety glasses when using the kit.
7. Dispose of all materials, containers and devices appropriately after use.

## Procedure

### Sample Preparation

#### For milk (dilution factor is 5):

1. Pipette 5mL of raw milk sample into a clean test tube.
2. Centrifuge milk sample with speed of 3000g at 10°C for 10 minutes.
3. Pipette 0.2mL of milk serum below the fat layer into a clean test tube.
4. Add 0.8mL of assay diluent into the milk serum and mix. The sample is ready for testing.

#### For milk powder (dilution factor is 25):

1. Weigh 1g of milk powder into a clean test tube.
2. Add 5mL of 50°C water to dissolve the milk powder.
3. Centrifuge the dissolved milk powder sample with speed of 3000g at 10°C for 10 minutes.
4. Pipette 0.2mL of milk serum below the fat layer into a clean test tube.
5. Add 0.8mL of assay diluent into the milk serum and mix. The sample is ready for testing.

### Assay

**Note:** All reagents and kit components must be at room temperature 18-30°C (64-86°F) before use. No more than 32 samples and standards total (4 test strips) should be run in one experiment when using an 8-channel pipettor. If an 8-channel pipettor is not used (i.e. using only single channel pipettes), it is recommended that no more than a total of 16 samples and standards (2 test strips) be run in any one experiment.

1. Place the appropriate number of Antibody Coated Microwell strips in a microwell strip holder. Return unused microwell strips to the foil pouch with the desiccant packet and reseal pouch.
2. Using a single channel pipettor, add **150 µL of each standard or sample** into the appropriate Antibody Coated Well. Use a fresh pipette tip for each standard or sample. **Note:** Make sure the pipette tip has been completely emptied.
3. Measure the required amount of Conjugate from the green-capped bottle (~70µL/well or 580µL/strip) and place in a separate container (e.g. reagent boat when using the 8-channel pipettor). Using an 8-channel pipette, dispense **50 µL of Conjugate** into each Antibody Coated Well, and mix each well by carefully pipetting it up and down 3 times
4. Incubate the wells for **30 minutes**.
5. Empty the contents of the microwell strips into a waste container. Wash by filling each microwell with distilled/deionized water, and then dumping the water from the microwell strips. Repeat this step 3 times for a total of 4 washes. **Note:** Take care not to dislodge the strips from the holder during the washing procedure.
6. Lay several layers of absorbent paper towels on a flat surface and tap microwell strips on towels to expel as much residual water as possible after the fourth wash. Dry the bottom of the microwells with a paper towel.
7. Measure the required amount of Substrate from the blue-capped bottle (~120 µL/well or 1 mL/strip) and dispense into a separate container (e.g. reagent boat for an 8-channel pipettor). Pipette **100 µL of the Substrate** into each microwell strip using an 8-channel pipettor. Incubate at room temperature for **20 minutes**.
8. Measure the required amount of Stop Solution from the red-capped bottle (~120 µL/well or 1 mL/strip) and dispense into a separate container (e.g. reagent boat for an 8-channel pipettor). Pipette **100 µL of Stop Solution** into each microwell strip using an 8-channel pipettor. The color should change from blue to yellow.

9. Read the strips with a microwell reader using a 450 nm filter. Record OD readings for each microwell.

**Note:** Air bubbles should be eliminated prior to reading strips as they may affect analytical results.

## Interpretation of the Results

**Semi-quantitative results:** semi-quantitative results can be obtained by simply comparing the color intensity of sample wells to the color intensity of standard wells before Stop Solution is added. Sample having less color intensity than a standard will have melamine concentration higher than the concentration in the standard, Sample having more color intensity than a standard will have melamine concentration lower than the standard.

**Quantitative results:** using the OD values expressed as a percentage of the OD value of the 0ppb standard, construct a semi-log response curve using the four standards. Since the amount of melamine in each standard is known, the unknowns can be measured by interpolation from this standard curve. Results can also be easily calculated using the Romer® Log/Logit spreadsheet that is provided (free of charge) upon request. If the Log/Logit regression model is used for results interpretation, the linearity coefficient ( $r^2$ ) of the calibration curve should be no less than 0.990. An OD value of less than 0.5 absorbance units for 0ppb standard may indicate deterioration of reagents.

If a sample contains melamine greater than the highest standard (500ppb x dilution factor), the filtered extract can be further diluted with sample diluent such that the diluted sample result is in the quantitation range and reanalyzed to obtain accurate result. The dilution factor must be included when the final result is calculated. If a sample contains melamine less than the lowest standard (20ppb x dilution factor), the result should be reported as "< (20ppb x dilution factor)".

## Performance Characteristics

**Quantitation Range:** 0.1 – 5.0ppm for milk  
0.5 – 25.0ppm for milk powder

**Limit of Detection:** 8.0ppb for milk  
150.0ppb for milk powder

**Recovery:** 102 – 126% for milk  
82 – 93% for milk powder

## Materials Supplied With Kit

- 96 antibody coated microwells (12 eight-well strips) in a microwell holder sealed in a foil pouch
- 4 vials of 2mL of each melamine standard (0, 20, 100 and 1000 ppb)
- 1 bottle of 7mL of melamine enzyme conjugate (green-capped bottle)
- 1 bottle of 14mL of substrate solution (blue-capped bottle)
- 1 bottle of 14mL of stop solution (red-capped bottle)
- 1 bottle of 100mL of sample diluent

## Materials Required But Not Provided With Kit

### Sample Preparation/Extraction Procedure

- Homogenizer or blender
- Balance
- Vortex
- Centrifuge with temperature control
- Centrifuge tube
- Test tube with a minimum 25mL capacity

### Assay Procedure

- \*8-channel or single channel pipettors capable of pipetting 150µL and 50µL with tips
- \*EQOLE1300: Timer
- \*COKAD1150: Wash bottle
- Absorbent paper towels

- \*3 reagent boats for use as reagent containers for an 8-channel pipettor
- \*Microwell reader with a 450nm filter (GIPSA approved readers: Stat Fax® 303 Plus manufactured by Awareness Technology Inc., or equivalent).

\*Items available from Romer Labs Singapore Pte Ltd

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