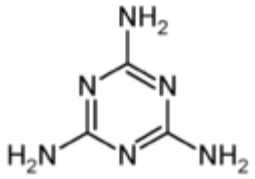
AgraQuant® Melamine Assay

Order #: COKAQ9300

Intended Use

The AgraQuant® Melamine 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, wheat gluten and pet food.



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.

Assay Principles

The AgraQuant® melamine assay is a direct competitive enzyme-linked immunosorbent assay (ELISA). Melamine is extracted from a sample by vortex or sonication. The extracted 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 laboratory grade water or wash solution. 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 / Extraction

For milk (dilution factor is 100):

1. Pipette 1mL of raw milk sample into clean test tube.
2. Add 9mL of 60/40 Methanol/water, and shake or vortex to mix well the content.
3. Dilute 1:10 of the sample solution with sample diluent (e.g., add 0.1mL of sample solution to 0.9mL sample diluent).

For milk powder (dilution factor is 500):

1. Weigh 1g of milk powder into a clean tube.
2. Add 5mL of 50ºC water to dissolve the milk powder.
3. Pipette 1mL of dissolved milk solution to a clean tube.
4. Add 9mL of 60/40 Methanol/water, and shake or vortex to mix well the content
5. Dilute 1:10 of the sample solution with sample diluent (e.g., add 0.1mL of sample solution to 0.9mL sample diluent)

For wheat gluten (dilution factor is 500):

1. Homogenize or blend wheat gluten sample.
2. Weigh out 0.2 g of homogenized sample into a clean tube.
3. Add 10mL of acidic 60/40 (v/v) methanol/water extraction solution and seal the tube. **Note:** acidic 60/40 (v/v) methanol/water is prepared by adding 1mL of 1N HCL into 100mL of 60% of methanol/water.
4. Vortex vigorously for 1 minute and sonicate for 1 minute to extract the sample.
5. Dilute the extract 1:10 with the sample diluent and mix (e.g., add 0.1mL of sample extract to 0.9mL of sample diluent).
6. Centrifuge the upper clear layer for 10 minute at a speed of 10,000rpm in a microcentrifuge
7. Filter the clear upper layer using a filter with a pore size of 0.45µm and collect the filtrate.
8. The sample is ready for testing without further preparation.

For moist pet food (dilution factor is 100):

1. Homogenize sample using a blender until it resembles a gritty pudding.
2. Weigh out 2 g of homogenized sample into a clean tube.
3. Add 10mL of 60/40 (v/v) methanol/water extraction solution and seal the tube, and vortex vigorously for 1 minute.
4. Vortex, and sonicate sample for 1 minute.
5. Vortex sample again for 1 minute, and then let sample stand for 5 minutes to allow layers to be separate.
6. Centrifuge the upper clear layer for 5 minutes at a speed of 10,000rpm using a microcentrifuge.
7. Filter the clear upper layer using a filter with a pore size of 0.45µm and collect the filtrate.
8. Dilute the clear extract 1:20 with the sample diluent (e.g., add 0.05mL of sample extract to 0.95mL of sample diluents)
9. The diluted sample is ready for testing without further preparation.

For dry pet food (dilution factor is 200):

1. Homogenize dry pet food using a blender or coffee grinder.
2. Weigh out 1 g of homogenized sample into a clean tube.
3. Add 10mL of 60/40 (v/v) methanol/water extraction solution and seal the tube.
4. Vortex vigorously for 1 minute, and sonicate for 1 minute.
5. Vortex sample again for 1 minute.
6. Let sample stand for 5 minutes to allow layers to be separate.
7. Centrifuge upper clear layer using a Microcentrifuge for 5 minutes at a speed of 10,000rpm.
8. Filter the clear upper layer using a filter with a pore size of 0.45µm and collect the filtrate.
9. Dilute the clear extract 1:20 with the sample diluent (e.g. add 0.05mL of sample extract to 0.95mL of sample diluents)
10. The diluted sample is ready for testing without further preparation.

Assay

Note: All reagents and kit components must be at room temperature 18-30°C (64-86°F) before use. No more than 48 samples and standards total (6 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 (for milk, milk powder and pet food samples) or wash solution (for wheat gluten), 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 **30 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 a sample contains melamine greater than the highest standard (500ppb x dilution factor), the filtered extract should 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

Range of quantitation: 10 – 250ppm for milk powder, wheat gluten
2 – 50ppm for milk, moist pet food
4 – 100ppm for dried pet food

Recovery:

Milk (for levels of 10 – 50ppm): 91 – 115%
Milk powder (for levels of 10 – 250ppm): 92 – 128%
Wheat gluten (for levels of 100 – 250ppm): 102 – 110%
Moist pet food (for levels of 10 – 40ppm): 83 – 98%
Dried pet food (for levels of 40 – 80ppm): 80 – 95%

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 500 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
- 1 bottle of 25mL of 20X Wash Solution Concentrate (blue-capped bottle)

Materials Required But Not Provided With Kit

Extraction Procedure

- Homogenizer or blender
- Balance
- Vortex
- Sonicator
- Microcentrifuge
- *filter with pore size of 0.45µm
- *EQOLE1050: Graduated cylinder: 100mL
- 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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Warranty

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