**Product: AflaCLEAN M1 SMART  
According to the matrix please select the appropriate method.**

**Extraction of milk / raw milk or UHT milk according to the offical AOAC method 2000.08:**

* 100 mL milk is prewarmed to room temperature. The milk is stirred to disperse the fat in the milk. The milk is centrifuged at 2000xg to separate fat from the residual milk. The thin fat layer is discarded.
* The milk is filtered through a plaited filter or glass fibre filter to remove any precipitations in the milk.
* The milk is diluted with equal amount of PBS buffer.
* The immunoaffinity column is adapted to room temperature and opened. A maximum of 20 mL diluted sample (represents 10 mL milk) is applied onto the immunoaffinity column.
* To wash the column, the sample reservoir is rinsed with 4 mL water. This solution is given onto the column.
* The washing efficiency could be increased by applying the washing solution stepwise (e.g. two times 2 mL).  
  Residual water is now removed by a gentle gas stream or vacuum.
* Elute with 0.4 mL of methanol; let the methanol act in the gel for 5 minutes.
  + To ensure correct elution volume, the sample vial could be balanced prior to collecting eluate. The sample vial should be balanced after collection of the eluate to calculate eluate weight. An aliquot of the sample is removed and the densitiy could be calculated. So the correct volume could be determined and finally the correct toxin concentration could be calculated.
* Dilute or concentrate eluate to your requirements and measure by HPLC.

**Extraction of milk powder according to the EN ISO 14501:2007:**

* 10 g of milk powder is put into a 250 mL beaker, add 50 mL water, prewarmed to 50 °C and stir to solve the test sample. In case that the sample is not solved completely the material could be warmed to 50 °C and the material is stirred frequently to solve completely.
* Allow the test solution to cool to between 20 °C and 25 °C.
* Quantitatively transfer the test solution to a 100 mL one-mark volumetric flask using small amounts of water. Dilute to the 100 mL mark with water. Filter enough of the reconstituted sample through filter paper or glass fibre filter or centrifuge it at a radial acceleration of at least 2000*g*. Collect at least 50 mL of the prepared milk powder sample. The prepared sample is diluted with an equal amount of PBS buffer (50 mL).
* The 20 mL aliquot (represents 10 mL reconstituted milk) is applied onto the immunoaffinity column, which need to be adapted to room temperature.
* To wash the column, the sample reservoir is rinsed with 4 mL water. This solution is given onto the column. The washing efficiency could be increased by applying the washing solution stepwise (e.g. two times 5 mL). Residual water is now removed by a gentle gas stream or vacuum.
* Elute with 0.4 mL of methanol; let the methanol act in the gel for 5 minutes.
  + To ensure correct elution volume, the sample vial could be balanced prior to collecting eluate. The sample vial should be balanced after collection of the eluate to calculate eluate weight. An aliquot of the sample is removed and the densitiy could be calculated. So the correct volume could be determined and finally the correct toxin concentration could be calculated.
* Dilute or concentrate eluate to your requirements and measure by HPLC.

**Extraction of aflatoxin M1 from cheese:**

* A sample of 10 g of cheese was cut into small pieces and blended for 2 min at high speed with 80 mL of methylene chloride and 7 g of diatomaceous earth. After washing further with 40 mL methylene chloride, the mixture was filtered (glass fibre filter) and pressed to released maximum amount of filtrate. The filtrates were combined and evaporated in a rotary evaporator at 40 ºC. The residue was dissolved in 1 mL methanol, 30 mL water and 50 mL n-hexane and transferred to a separating funnel.
* The water phase (lower layer) is collected.
* The n-hexane phase was then washed twice with 10 mL water and the water phase was also collected. Subsequently both water phases were homogenised and then applied to an immunoaffinity column. Make sure that no n-Hexane is applied onto the immunoaffinity column.
* To wash the column, the sample reservoir is rinsed with 10 mL water. This solution is given onto the column.
* The washing efficiency could be increased by applying the washing solution stepwise (e.g. two times 5 mL). Residual water is now removed by a gentle gas stream or vacuum.
* Elute with 0.4 mL of methanol; let the methanol act in the gel for 5 minutes.
  + To ensure correct elution volume, the sample vial could be balanced prior to collecting eluate. The sample vial should be balanced after collection of the eluate to calculate eluate weight. An aliquot of the sample is removed and the densitiy could be calculated. So the correct volume could be determined and finally the correct toxin concentration could be calculated.
* Dilute or concentrate eluate to your requirements and measure by HPLC.

**If you have any questions, please contact:** mycotoxins@LCTech.de

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