**Product: AflaCLEAN M1 Select  
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 immunoaffinity column is adapted to room temperature and opened. A maximum of 50 mL milk 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.
* 2 mL of methanol are applied onto the open column. Wait until the methanol has reached the lower Luer exit of the column. The column is immediately closed and incubated for 5 minutes to efficently break the analyt-antibody-binding. After the column is reopened, the eluate is collected in a 2 mL measuring cylinder by flushing air through the column. Residual eluate could be collected in the 2 mL measuring cylinder. Finally the 2 mL are adjusted by adding methanol.
* 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 2 000g. Collect at least 50 mL of the prepared milk powder sample.
* The 50 mL aliquot is applied onto the immunoaffinity column, which need to be adapted to room temperature.
* 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.
* 2 mL of methanol are applied onto the open column. Wait until the methanol has reached the lower Luer exit of the column. The column is immediately closed and incubated for 5 minutes to efficently break the analyt-antibody-binding. After the column is reopened, the eluate is collected in a 2 mL measuring cylinder by flushing air through the column. Residual eluate could be collected in the 2 mL measuring cylinder. Finally the 2 mL are adjusted by adding methanol.
* 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.
* 2 mL of methanol are applied onto the open column. Wait until the methanol has reached the lower Luer exit of the column. The column is immediately closed and incubated for   
  5 minutes, to efficently break the analyt-antibody-binding. After the column is reopened, the eluate is collected in a 2 mL measuring cylinder by flushing air through the column. Residual eluate could be collected in the 2 mL measuring cylinder. Finally the 2 mL are adjusted by adding methanol.
* 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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