**Product: AflaCLEAN SMART**

**According to the matrix please select the appropriate method.**

**1 For non fatty matrices recommended e.g. maize:**

* Extract 20 g of sample with 100 mL (80 % methanol : 20 % water) in a blender jar at high speed for five minutes.
* Pass the extract through a plaited filter.
* Add 14 mL of the purified extract to 86 mL PBS buffer (pH 7.2).
* Continue with 4.

**2 For fatty matrices recommended e.g. peanut, hazelnut and pistachio paste:**

* Add 2.0 g of NaCl to 20 g of sample.
* Extract with 100 mL (80 % methanol : 20 % water) and 50 mL of n-hexane in a blender jar at high speed for five minutes.
* Pass the extract through a plaited filter  
  *(Note: if there is a separation of phases to be found, the lower liquid phase is used for the following steps). To accelerate phase separation the extract could be centrifuged at 1000xg for 5 min.*
* Add 14 mL of the purified extract to 86 mL PBS buffer (pH 7.2).
* Continue with 4.

**3 For spices and animal feed e.g. black pepper, nutmeg, turmeric, chilli, pet food, animal feed:**

* Add 2.0 g NaCl to the sample.
* Extract with 100 mL of methanol: water (8:2) and 50 mL of n-hexane in a blender jar at high speed for three minutes.
* Pass the extract through a plaited filter.  
  *(Note: If there is a separation of phases to be found, the lower liquid phase is used for the following steps). To accelerate phase separation the extract could be centrifuged at 1000xg for 5 min.*
* Add 1 mL of the purified extract to 6 mL PBS buffer containing   
  8 % Tween20.
* Continue with 4.

**4 Immunoaffinity chromatography procedure:**

* The sample is recommended to be passed through a whatman filter to remove residual turbidity.
* Take 0 - 10 mL of the diluted extract (depending on the extraction procedure and the sensitivity of detection), for spices (e.g. black pepper, cumin, turmeric, ginger, coriander) a maximum of 2.8 mL could be applied onto the AflaCLEAN SMART column. It is indispensable to maintain a maximum flow rate of 3 mL/min. 10 mL diluted extract (using protocol 1 or 2) represent 0.28 g matrix. 2.8 mL diluted extract (using protocol 3) represent 0.08 g matrix.
* To wash the column, pass 2 mL of distilled water through the column with a maximum flow rate of 3 mL/min.
* Carefully remove the residual water by flushing air through the column.
* 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 directly by HPLC; alternatively, carefully concentrate to dryness and store cool and in the dark.

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

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