**Product: Afla-OtaCLEAN, 3 mL, widebore**

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

**1 For non fatty matrices recommended e.g. wheat, barley, 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. nuts, paprika, chilli, and pistachio paste:**

* Add 2.0 g of NaCl to 20 g of sample.
* Extract with 100 mL of methanol: water (8:2) 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 recommended e.g. black pepper, coriander, cumin, turmeric, and ginger:**

* Add 2.0 g of NaCl to 20 g of sample.
* Extract with 100 mL of methanol : water (8:2) 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 5 mL of the purified extract to 30 mL PBS buffer (pH 7.2)   
  containing 8 % Tween20.
* Continue with 4.

**4 Immunoaffinity chromatography procedure:**

* The sample is recommended to be passed through a 0.2 µ syringe filter to remove residual turbidity.
* Take 5 - 50 mL of the diluted extract (depending on the extraction procedure and the sensitivity of detection) and pass it through the Afla-OtaCLEAN column. For spices (e.g. black pepper, cumin, turmeric, ginger, coriander) a maximum of 14 mL could be applied onto the Afla-OtaCLEAN column. A gentle vacuum or overpressure may be used in all steps passing liquid through the column; nevertheless, it is indispensable to maintain a maximum flow rate of 2 mL/min. 50 mL diluted extract (using protocol 1 or 2) represent 1.4 g matrix. 14 mL diluted extract (using protocol 3) represent 0.4 g matrix.
* To wash the column, pass 10 mL of distilled water through the column.
* Carefully remove the residual water in the column.
* Elute with at least 2 times 1 mL of methanol; let the first addition of methanol act on the gel for 5 minutes.
* 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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