**Product: OtaCLEAN SMART**

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

**1 For non fatty matrices recommended e.g. wheat, corn, rice:**

* Extract 20 g of sample with 100 mL (80 % methanol : 20 % water) in a blender jar at high speed for three minutes.
* Pass the extract through a plaited filter.
* Add 3 mL of the purified extract to 12 mL PBS buffer (pH 7.2).
* Continue with 7.  
  *(Note: If there is a strong precipitation by mixing with PBS buffer, a further filter or centrifuge step is highly recommended before passing through the IAC column).*

**2 For fatty matrices recommended e.g. peanut, raisins, seed:**

* 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 3 mL of the purified extract to 12 mL PBS buffer (pH 7.2).
* Continue with 7.

**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 7.

**4 For coffee matrices recommended e.g. coffee beans, instant coffee:**

* *5.0 g of the sample were homogenized and solved in 40 mL   
  (50 % methanol / 50%, 3 % NaHCO3). 10 mL of this solution are mixed thoroughly with 10 mL of dichloromethane for 5 min.The upper liquid phase was mixed with 10 mL dichloromethane for 5 min.*
* *(Note: If there is a separation of phases to be found, the upper liquid phase is used  
   for the following steps). To accelerate phase separation the extract could be centrifuged at 1000xg for 5 min.*
* Add 0.8 mL of the purified extract to 19.2 mL PBS buffer (pH 7.2).
* Continue with 7.

**5 For wine matrices recommended e.g. red wine, white wine:**

* 10 mL of the sample was mixed with 10 mL (1 % PEG, 5 % NaHCO3) for 3 min.
* The sample is passed through a plaited filter to remove precipitations.
* 3 mL of the sample was diluted with 12 mL PBS (pH 7.2).
* Continue with 7.

**6 For beer matrices recommended:**

* 20 mL of the sample were degassed by sonication.
* 8 mL of 3 % NaHCO3 solution was added and mixed well.
* The sample is passed through a plaited filter to remove precipitations and suspended particles.
* 3 mL of the sample was diluted with 12 mL PBS (pH 7.2).
* Continue with 7.

**7 Immunoaffinity chromatography procedure:**

* The diluted extract is passed through a 0.2 µ syringe filter.
* 1-10 mL (depending on the sensitivity of the detection) is applied on the OtaCLEAN SMART 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 1.5 mL/min.   
  (10 mL diluted extract (using protocol 1 or 2) represent 0.4 g matrix.  
  2.8 mL diluted extract (maximum applicable volume for this extraction using protocol 3) represent 0.08 g matrix.   
  10 mL diluted extract (using protocol 4) represent 0.05 g matrix.   
  10 mL diluted extract (using protocol 5) represent 1 g matrix).  
  10 mL diluted extract (using protocol 6) represent 1.428571 g matrix).
* To wash the column, pass 2 mL of distilled water through the column, which could be used to wash residual sample material from the reservoir.
* Carefully remove the residual water in 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 with fluorescence detection. The use of a post-column derivatization is recommended!

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

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