IAC-Column-Handling:

Question	Answer
What should be observed before use?	The columns should be brought to room temperature before use so that the binding function of the antibody is optimal.
What methanol contents do the antibodies tolerate?	Too high methanol contents are to be avoided in the applied samples, the specifications in relation to the solvent concentrations: AflaCLEAN /AflaCLEAN Select 11.2% Afla-OtaCLEAN 11.2% OtaCLEAN 16 % These should not be exceeded as partial denaturation of the antibodies will negatively affect the binding efficiency.
What are the maximum volumes that can be loaded onto the columns?	The sample volumes for the 3 mL columns of a maximum of 50 mL or 14 mL for the PBS/Tween protocols should not be exceeded, as the binding properties are more stressed by matrix components with larger sample volumes. For the SMART columns, sample volumes of 10 mL (AflaCLEAN SMART, OtaCLEAN SMART) to 20 mL (AflaCLEAN M1 SMART) are recommended.
Which matrix quantities can be applied?	AflaCLEAN /AflaCLEAN Select 0.4 grams (Tween) - 1.4 grams AflaCLEAN SMART 0.08 grams (Tween) - 0.28 grams Afla-OtaCLEAN 0.4 grams (Tween) - 1.4 grams OtaCLEAN 0.4 grams (Tween) - 2 grams (solid matrices) - 8 mL (liquid matrices) OtaCLEAN SMART 0.08 grams (Tween) - 0.4 grams (solid matrices) - 1.428 mL (liquid matrices) AflaCLEAN M1 Select (50 mL milk; 5 grams milk powder)) AflaCLEAN M1 SMART (10 mL milk, 1 gram milk powder) ZeaCLEAN SMART (0.5 gram matrix) DONeX (4 grams of matrix)
How do I start the column clean-up process?	The lids of the columns and the Luer cap at the column outlet should be opened to load samples, it is not necessary to remove the storage buffer before loading the sample, the sample can be applied directly to the remaining storage buffer in the column.
The column is not running, how can I start the process?	In case the column does not run on its own, tapping can remove possible air bubbles from the frits, using a syringe adapter or a vacuum manifold, an air bubble located in the column outlet can be removed from the column by applying slight pressure, the column should then start running on its own.
	When working in a vacuum manifold, removing the air bubbles is helpful, but not absolutely necessary, as the vacuum will pull them out of the column.

Question	Answer
How can I avoid matrix interference in the eluate?	After loading the sample onto the column, make sure that the sample has passed the column before applying the wash solution (deionised water). Mixing the sample and the wash solution only affects the purity of the eluate, but has no effect on the toxin concentration.
Which steps have to be taken when eluting the column?	To elute the analytes from the column, the following steps should be followed. 1. dry the column bed to remove water residues and to counteract falsification of the elution volume. 2. the eluent (1 mL methanol) should be added to the column bed, this should flow into the column bed to completely denature the antibody. 3. the eluent should be allowed to soak into the column bed for 5 minutes before this is collected. 4. elute with another mL of methanol, no 5 minute waiting time is required. 5. eluate residues are pressed out of the column by means of a syringe filter and also collected. 6. The eluate fractions are combined and represent the total eluate, which can be analysed by liquid chromatography.

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