

## Application note:

**AgraQuant® Plus Almond (COKAL0748F)**

**AgraQuant® Plus Casein (COKAL1248F)**

**AgraQuant® Plus Cashew (COKAL3148F)**

**AgraQuant® Plus Egg (COKAL1848F)**

**AgraQuant® Plus Hazelnut (COKAL0348F)**

**AgraQuant® Plus Macadamia nut (COKAL1648F)**

**AgraQuant® Plus Mustard (COKAL2148F)**

**AgraQuant® Plus Peanut (COKAL0148F)**

**AgraQuant® Plus Pistachio (COKAL2748F)**

**AgraQuant® Plus Sesame (COKAL1948F)**

## Procedure for environmental sampling using a sponge

### Sample Preparation / Extraction

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#### Sampling

1. Moisten dry sponges with water and rub on the surface using horizontal and vertical movements. Sponges that are moist can be used immediately.
2. Transfer the sponge to a 50 mL graded centrifuge tube.

*Note:* The sponge may need to be cut into small pieces (e.g. with a knife or scissors).

### Extraction

1. Bring the required amount of distilled or deionised water (20 mL per sample plus a reserve) to the boil in a kettle, or heat to **80-100 °C** in a water-bath. For **AgraQuant® Plus Egg** heat to **60 °C**.
2. Add one capsule each of extraction additives 1 and 2 to the centrifuge tube containing the sponge.
3. Top up to the 20 mL mark with the hot, but not boiling water from Step 1 (or add 20 mL). Tightly close the centrifuge tube and shake vigorously for **15 seconds**.
4. Transfer an aliquot to a 1.5 or 2 mL reaction vial and centrifuge at  $\geq 8,000$  g for 5 min. Alternatively, filter through a pleated filter paper. If required, dilute with specially prepared sample dilution buffer (=sample dilution factor F). Leave the sample extracts to cool down to room temperature before using in ELISA.

### Sample dilution buffer:

Make as described in Steps 1 – 4, but without sponge.

### Results Interpretation for environmental samples (sponge):

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The sponge's whole almond content is calculated using Equation (1). Conversion to almond protein content is done based on Equation (2).

$$(1) \text{whole almond } [\mu\text{g/sponge}] = \text{value from the standard curve} \times F$$

$$(2) \text{almond protein } [\mu\text{g/sponge}] = \text{whole almond } [\mu\text{g/sponge}] \times 0.2$$

The sponge's absolute casein content is calculated using Equation (3). Conversion to the skim milk powder content is done based on Equation (4).

$$(3) \text{casein } [\mu\text{g/sponge}] = \text{value from the standard curve} \times F$$

$$(4) \text{skim milk powder } [\mu\text{g/sponge}] = \text{casein } [\mu\text{g/sponge}] \times 3.5$$

The sponge's absolute cashew content is calculated using Equation (5). Conversion to cashew protein content is done based on Equation (6).

$$(5) \text{total cashew } [\mu\text{g/sponge}] = \text{value from the standard curve} \times F$$

$$(6) \text{cashew protein } [\mu\text{g/sponge}] = \text{total cashew } [\mu\text{g/sponge}] \times 0.18$$

The whole egg powder content is calculated using Equation (7).

$$(7) \text{whole egg powder } [\mu\text{g/l}] = \text{value from standard curve} \times F \times 100$$

The sponge's whole macadamia content is calculated using Equation (8). Conversion to macadamia protein content is done based on Equation (9).

$$(8) \text{ whole macadamia } [\mu\text{g/sponge}] = \text{value from the standard curve} \times F$$

$$(9) \text{ macadamia protein } [\mu\text{g/sponge}] = \text{whole macadamia } [\mu\text{g/sponge}] \times 0.13$$

The sponge's absolute mustard content is calculated using Equation (10). Conversion to mustard protein content is done based on Equation (11).

$$(10) \text{ total mustard } [\mu\text{g/sponge}] = \text{value from the standard curve} \times F$$

$$(11) \text{ mustard protein } [\mu\text{g/sponge}] = \text{total mustard } [\mu\text{g/sponge}] \times 0.12$$

The sponge's whole peanut content is calculated using Equation (12). Conversion to peanut protein content is done based on Equation (13).

$$(12) \text{ whole peanut } [\mu\text{g/sponge}] = \text{value from the standard curve} \times F$$

$$(13) \text{ peanut protein } [\mu\text{g/sponge}] = \text{whole peanut } [\mu\text{g/sponge}] \times 0.25$$

The sponge's absolute pistachio content is calculated using Equation (14). Conversion to the protein content is done based on Equation (15).

$$(14) \text{ total pistachio } [\mu\text{g/sponge}] = \text{value from the standard curve} \times F$$

$$(15) \text{ pistachio protein } [\mu\text{g/sponge}] = \text{total pistachio } [\mu\text{g/sponge}] \times 0.18$$

The sponge's absolute sesame content is calculated using Equation (16). Conversion to the protein content is done based on Equation (17).

$$(16) \text{ total sesame } [\mu\text{g/sponge}] = \text{value from the standard curve} \times F$$

$$(17) \text{ sesame protein } [\mu\text{g/sponge}] = \text{total sesame } [\mu\text{g/sponge}] \times 0.18$$

### Limit of Detection

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0.5 µg/sponge whole almond  
 0.2 µg/sponge Casein  
 1 µg/sponge total cashew  
 0.5 µg/sponge whole egg powder  
 1 µg/sponge whole hazelnut  
 1 µg/sponge whole macadamia  
 0.5 µg/sponge total mustard  
 0.5 µg/sponge whole peanut  
 1 µg/sponge whole pistachio  
 1 µg/sponge whole sesame

### Limit of Quantification

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1 µg/sponge whole almond  
 1 µg/sponge Casein  
 1 µg/sponge total cashew  
 1 µg/sponge whole egg powder  
 1 µg/sponge whole hazelnut  
 1 µg/sponge whole macadamia  
 1 µg/sponge total mustard  
 1 µg/sponge whole peanut  
 1 µg/sponge whole pistachio  
 1 µg/sponge whole sesame

Since AgraQuant® Plus Almond (COKAL0748F), AgraQuant® Plus Casein (COKAL1248F), AgraQuant® Plus Cashew (COKAL3148F), AgraQuant® Plus Hazelnut (COKAL0348F), AgraQuant® Plus Macadamia nut (COKAL1648F), AgraQuant® Plus Mustard (COKAL2148F), AgraQuant® Plus Peanut (COKAL0148F) and AgraQuant® Plus Sesame use the same extraction additives and extraction temperature of 80-100°C, extracts obtained with one of those kits can be used to screen for all eight allergens with the respective kit (it is advised to use the extracts immediately and not to store them in the fridge or freezer, since there is no validation data available at the moment backing this up).