

Application note:

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)

AgraQuant® Plus Sesame (COKAL1948F)

One extraction method for eight AgraQuant® Plus kits

Since all seven kits 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 seven 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).



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Same sample preparation procedure for all eight kits

I. Procedure for foodstuffs:

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** using a water-bath.
2. Accurately weigh 1 g of sample into a 50 mL centrifuge tube and add one capsule each of extraction additives 1 and 2.
3. Add 20 mL hot, but not boiling water from Step 1. Tightly close the centrifuge tube and shake vigorously for **15 seconds** (until sample and capsules are dissolved).
4. Transfer an aliquot to a 1.5 or 2 mL centrifuge tube and centrifuge at $\geq 8,000$ g for 5 min, use the supernatant for testing. Alternatively, filter through a pleated filter paper and use the filtrate in the test. If required, dilute with specially prepared sample dilution buffer (=sample dilution factor F). Leave the sample extracts to cool down to room temperature before carrying out the ELISA.

Sample dilution buffer:

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

II. Procedure for environmental samples (swabs):

Sampling

1. Moisten swab with water and rub on the surface using horizontal movements, turning the swab while doing so. Repeat procedure with vertical movements.
2. Break off or cut off swab and transfer to a 10 mL graded centrifuge tube.

Extraction

1. Bring the required amount of distilled or deionised water (at least 20 mL) to the boil in a kettle, or heat to **80-100 °C** in a water-bath.
2. Add one capsule each of extraction additives 1 and 2 per 20 mL water, and shake vigorously for 15 seconds (until the capsules are dissolved).
3. Add 2 mL of the extraction buffer thus prepared into the 10 mL centrifuge tube containing the swab, close tightly and shake vigorously for **15 seconds**.

4. Transfer the liquid into a 2 mL reaction vial and centrifuge at $\geq 8,000$ g for 5 min. Alternatively, filtrate through a pleated filter. If required, dilute with the extraction buffer from Step 2 (=sample dilution factor F). Leave the sample extracts to cool down to room temperature before using in ELISA.

III. Procedure for rinse water:

Sample preparation

Dilute the rinse water (pH 4-9) 1:2 in a suitable vial using sample dilution buffer (prepare as described in Section III., but without sponge), e.g. dispense 0.5 mL rinse water into the vial, add 0.5 mL sample dilution buffer and shake. Dilute with the same buffer if required (= sample dilution factor F).