

# Allergen extraction from food samples using the Precellys® Evolution

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## CONTEXT

In this study, the objective is the identification of allergens recognized by the IgE antibodies from allergic patients. Genclis SAS used the Precellys® Evolution to homogenize food samples for protein extraction. The quality of proteins extracted with the Precellys® Evolution was compared by Coomassie blue staining and by western blot to two other homogenizers: the IKA Ultra-Turrax® dispenser and the Awel Mixwell® blender.

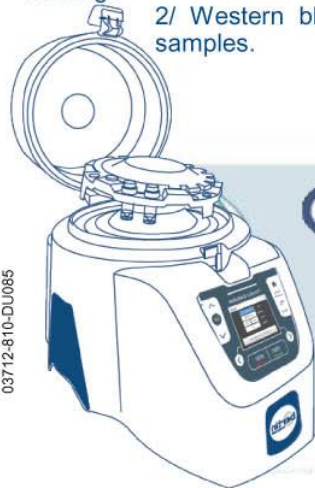
## MATERIAL

- Precellys® Evolution vs IKA Ultra-Turrax® & Awel Mixwell®.
- Precellys® lysing kit: 15mL empty tube (KT03961-1-406.15) + 2.4g CK28 beads (KT03961-1-102.BK) + 4g CK14 beads (KT03961-1-103.BK) + 4 CK50 beads (KT03961-1-106.BK).
- Sample: 1.2g of dog biscuits, 1.6g of cashew nuts and 1.6g of frozen green beans.
- Buffer: 8mL of PBS pH7.4 (Sigma).

## PROTOCOL

- Precellys® Evolution: 6500rpm, 2x30sec, 30sec break.
- IKA Ultra-Turrax® dispenser: approx. 2x30sec.
- Awel Mixwell® blender: approx. 5 min.
- Analysis: 1/ Protein extracts (15 µg of dog biscuits and cashew nuts; 30µL of green beans) were loaded on a 12% polyacrylamide gel and separated using SDS-PAGE electrophoresis, followed by Coomassie blue staining.

2/ Western blot analysis on cashew nut samples.



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## CONCLUSION

Precellys® Evolution homogenizer is suitable and convenient for allergen extraction from large food samples. The Precellys® Evolution homogenizer is superior to the two other blenders tested regarding the length of homogenization time. One to six 15mL samples can be homogenized at the same time and in less than two minutes. And, more time is saved by eliminating the wash step in between samples. Moreover, the Precellys® Evolution avoids the problem of protein cross-contamination between samples with the use of individual tubes.

## RESULTS

The protein profiles of the 3 samples were compared by Coomassie blue staining (Figure 1) and by western blot for the cashew nut samples only (Figure 2). The cashew nut allergens were recognized by specific IgE antibodies, as observed by western blotting. Similar protein and allergenicity profiles were obtained with Precellys® Evolution compared to the two other homogenizers compared in this study.

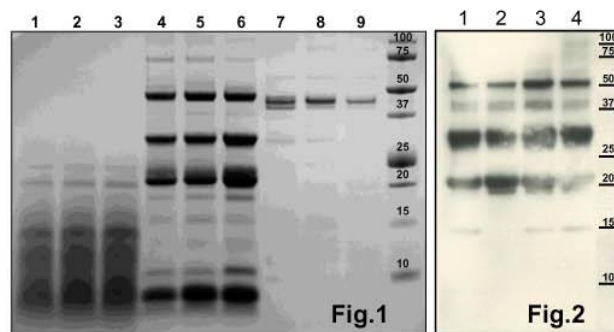


Figure 1: Comparison by Coomassie staining of the food extracts homogenized with the Precellys Evolution® (PE), the IKA Ultra-Turrax® dispenser (IK) and the Awel Mixwell® blender (AM). Lane 1 - dog biscuits/PE; 2 - dog biscuits/IK; 3 - dog biscuits/AM; 4 - cashew nuts/PE; 5 - cashew nuts/IK; 6 - cashew nuts/AM; 7 - green beans/PE; 8 - green beans/IK; 9 - green beans/AM.

Figure 2: IgE reactivity was compared in cashew nut extracts by western blot after homogenization with the 3 instruments. Samples were loaded onto a 12% polyacrylamide gel, and separated by SDS PAGE electrophoresis, followed by transfer to PVDF membrane. The membrane was incubated with the serum of a patient allergic to cashews, followed by probing with a secondary anti-Human IgE HRP-conjugated antibody. Lane 1 - cashew nuts/PE; 2 - cashew nuts/PE; 3 - cashew nuts/IK; 4 - cashew nuts/AM.



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