Natural Method for Allergen Identification using FastPrep-24™ 5G Technology.

Pollen

CASE STUDY

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Processus de Combustion et de l'Atmosphère, Lille. Institut Pasteur, département Infection & Epidémiologie, Paris. 2015

Overview

Keywords: Allergen, IgE immunoreactivity, pollen homogenization, hypersensitivity community, DNA extraction

Aim of the study: Identification of a fast method for protein extraction from pollen grains

Application: Western blot analysis

Sample name: Birch, Nettle, Wall Pellitory pollens

Sample type: Pollen

Material: FastPrep-24™ 5G instrument, CoolPrep adapter, 2 mL Lysing Matrix C & E tubes

Buffer: PBS

Protocol and Parameters

Incubation Method

1. Add 50 mg of pollen and 500 µL of PBS in a tube
2. Place the tube in a shaker for 18 hours in cold room
3. Centrifuge the suspension 20 mins at 18,000 x g, 4°C
4. Keep the supernatant at -20°C prior to analysis

Grinding Method

1. Add 50 mg of pollen and 500 µL of PBS to a 2 mL Lysing Matrix C or E tube.
2. Load Lysing Matrix tubes in a CoolPrep Adapter containing dry ice.
3. Process with the FastPrep-24 5G: 40 sec at a speed setting of 6.0 m/s.
4. Centrifuge the Lysing Matrix tubes 20 mins at 18,000 x g, 4°C to pellet debris.
5. Keep the supernatant at -20°C prior to analysis

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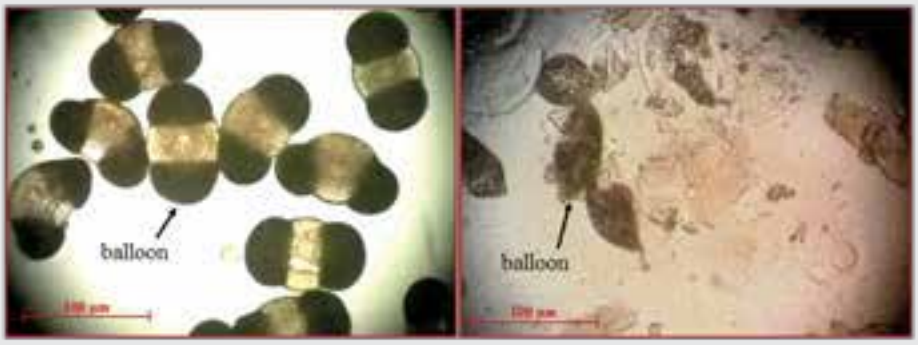
Conclusion

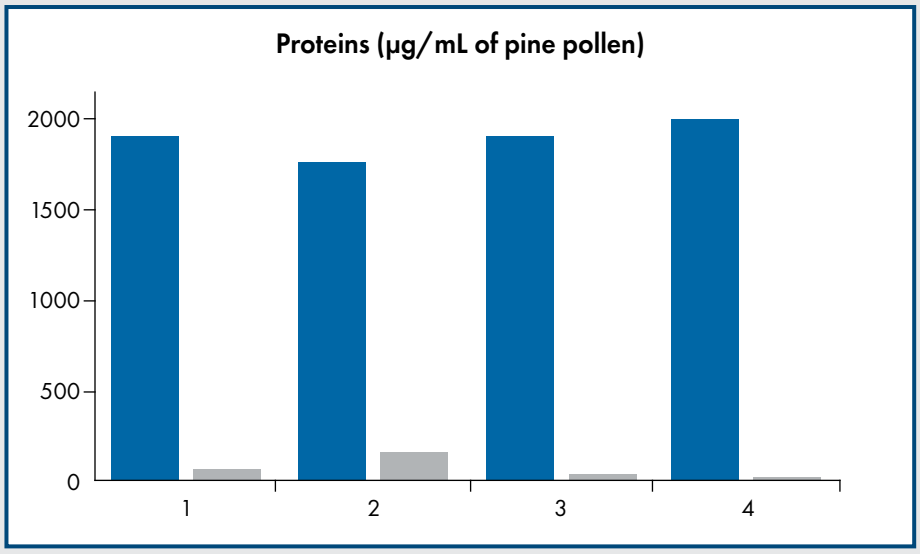
Protein extraction from pollen samples with the FastPrep-24™ 5G showed to be highly effective compared to the standard method based on overnight incubation. The effectiveness of the FastPrep method is quantitative, resulting in higher protein yields, as well as qualitative, as evidenced by a wide variety composition of protein extracts. The FastPrep system is a powerful tool to rapidly generate protein extracts with high reproducibility, ready for electrophoresis (SDS-PAGE) analysis. IgE immunoreactivity is conserved in protein extracted with the FastPrep-24™ 5G instrument.

Total destruction of the pollen grain structure with FastPrep-24™ 5G instrument and Lysing Matrix C



Optical microscope observation of pine pollen (x200) before (left) and after grinding (right) with the FastPrep-24™ 5G System.

Up to 2 mg/mL of protein extracted with FastPrep-24™ 5G System



Comparison of 8 pine pollen protein extracts obtained by standard or FastPrep method. Experimenst were repeated 4 times using 4 different pollen batches. Protein concentration was determined using Bradford assay.

FastPrep-24™

Incubation/Rotation

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