MP Biomedicals

Case study - *Pollen*

Health

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

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Introduction

Allergy is a hypersensitivity disorder of the immune system. According to epidemiological studies, at present, 20-30% of the population in many countries around the world suffers from allergies, and this percentage is growing trend.
Pollen are significant sources of clinically relevant out door aeroallergens, recognized as both a major trigger for, and cause of, allergic respiratory diseases.
Allergens are proteins with a broad range of molecular weights (5-50 kDa) exhibiting different features of solubility and stability, able to cause IgE-mediated hypersensitivity after contact with the immune system. The development of new types of allergy treatments needs diverse and well-characterized allergenic source materials. This study describes an effective method for allergen characterization.

Overview

- **Keyword:** Allergen, IgE immunoreactivity, pollen homogenization, hypersensitivity community, DNA extraction
- **Aim of the study:** identification of 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

1- Incubation method

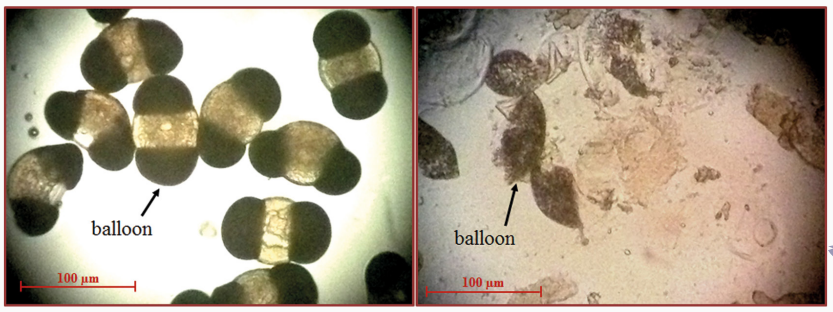
- Add 50 mg of pollen and 500 µl of PBS in a tube
- Place the tube in a shaker for 18 hours, in cold room
- Centrifuge the suspension 20 mins at 18 000 x g, 4 °C
- Keep the supernatant at -20 °C prior to analyses

2- Grinding method

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

Results

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



(FastPrep-24™ 5G, MP Biomedicals, CoolPrep, 40s, 6m/s)

Optical microscope observation of pine pollen (X 200) before and after grinding with the FastPrep-24™ 5G System.

***Left:** pollen grain before grinding.*

***Right:** homogenized pollen grain with FastPrep-24™ 5G, 40s at speed 6 m/s with Lysing Matrix C.*

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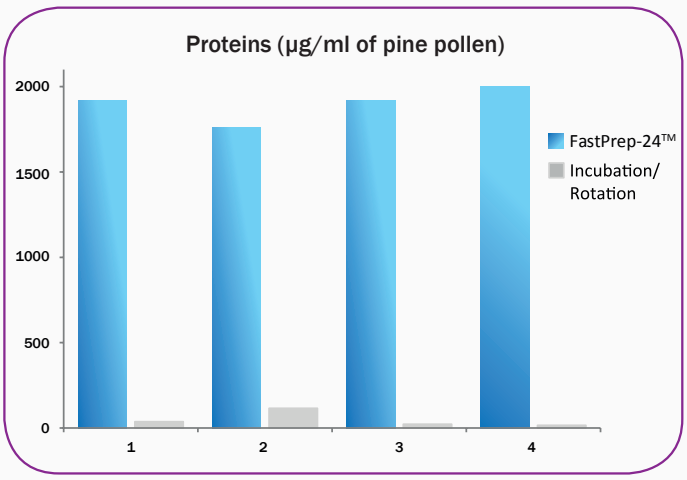
Results

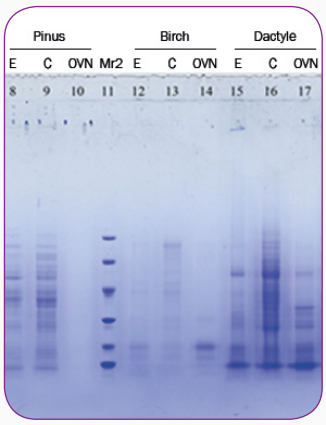
Up to 2 mg/ml of protein extracted with FastPrep-24™ 5G System



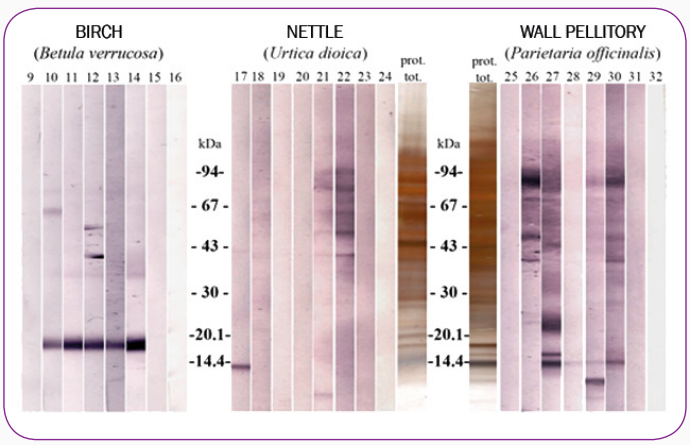
Comparison of 8 pine pollen protein extracts obtained by standard or FastPrep® method. Experience is repeated 4 times using 4 different pollen batches. Protein concentration is determined using Bradford assay.

Effective protein extraction, with the FastPrep-24™ 5G System, for all the tested pollen

Comparison of protein extraction with standard (OVN) or FastPrep method, using Lysing Matrix C or E. Coomassie blue gel staining.

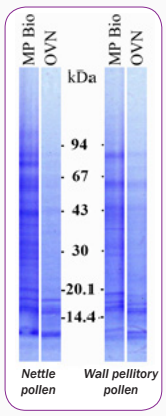


IgE immunoreactivity conservation for pollen extracts



Conserved IgE immunoreactivity in pollen extracts obtained with FastPrep-24™ 5G instrument. Left, IgE immunoreactivity of 6 birch pollen allergic patients tested against birch pollen protein (9-14 strips). Middle and right, IgE immunoreactivity of 12 patients, allergic to herbaceous pollen, tested against nettle and wall pellitory pollen protein (17-22 & 25-30). Relative masses expressed in kDa. 15, 16, 23, 24, 31 & 32 strips correspond to negative controls.

Wide variety composition of protein extracted with FastPrep® method



Protein profiles comparison of nettle and wall pellitory pollen obtained by FastPrep-24™ 5G (MP Bio) homogenization or by overnight incubation (OVN). The 4 extracts are used without dilution or concentration. SDS-PAGE 8-18 %. Coomassie blue gel staining.

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, **higher protein yield**, and qualitative, wide variety composition of protein extracts.
- The FastPrep® system is a powerful tool to get rapidly and with a very **high reproducibility** protein extracts ready for electrophoresis (SDS-PAGE) analysis.
- IgE immunoreactivity is conserved in protein extracted with the FastPrep-24™ 5G instrument.