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Letter to the Editor



# Novel Sensitive, Two-site ELISA for the Quantification of Der f 1 Using Monoclonal Antibodies

**Ji Tae Kim**,[1,2] **Jongsun Lee**,[3] **Jie Eun Yuk**,[3] **Hangyeol Song**,[3] **Hyunho Kim**,[1] **Sung Hyun Kim**,[1] **Dong Jun Kim**,[1] **Yeji Shin**,[1] **Deug-Chan Lee**,[2] **Kyoung Yong Jeong**,[1,3*] **Jung-Won Park**[1,3*]

[1]Prolagen, Seoul, Korea
[2]Department of Biomedical Technology, Kangwon National University, Chuncheon, Korea
[3]Department of Internal Medicine, Institute of Allergy, Yonsei University College of Medicine, Seoul, Korea



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**Correspondence to**
**Jung-Won Park, MD, PhD**
Department of Internal Medicine, Institute of Allergy, Yonsei University College of Medicine, 50-1 Yonsei-ro, Seodaemun-gu, Seoul 03722, Korea.
Tel: +82-2-2228-1961
Fax: +82-2-362-8647
E-mail: parkjw@yuhs.ac

**Kyoung Yong Jeong, PhD**
Department of Internal Medicine, Institute of Allergy, Yonsei University College of Medicine, 50-1 Yonsei-ro, Seodaemun-gu, Seoul 03722, Korea.
Tel: +82-2-2228-0808
Fax: +82-2-362-8647
E-mail: jeongky@yuhs.ac



**ORCID iDs**
Ji Tae Kim
https://orcid.org/0000-0002-8690-358X
Jongsun Lee
https://orcid.org/0000-0003-1526-2241

To the Editor,

The efficacy of allergen immunotherapy depends on the quality of allergen extracts. Currently, biological methods based on intradermal skin tests or skin prick tests are utilized for the preparation and standardization of reference allergen extracts.[1] A competitive inhibition assay is normally used for *in vitro* comparison of allergenic potency and standardization of new preparations.[2] However, quantification of major allergen content is needed for better allergen standardization. The concentration of major allergen in an extract is proportional to its allergenic activity, and maintenance doses (5 to 20 μg/dose, 50 to 250 μg/yr) of the major allergen are necessary for effective subcutaneous immunotherapy.[3] The Japanese Society of Allergology determined allergenic potency based on Der p 1 and Der f 1 content, concluding that 38.5 μg/mL of Der 1 is equivalent to 100,000 JAU/mL.[4]

A monoclonal antibody (mAb)-based sandwich enzyme-linked immunosorbent assay (ELISA) for the quantification of Der f 1 and Der p 1 was first developed in 1989[5] and is currently in wide use. More recently, tandem mass spectrometry has begun to be utilized for allergen quantification.[6] However, its use is limited due to the need for expensive instruments and highly trained staffs for routine use.

The study presented here was undertaken to develop a new sensitive, two-site ELISA for quantification of Der f 1. Hybridomas producing mAbs were prepared by immunization with recombinant (r) Der f 1, which was produced in *Pichia* as previously described.[7] First, 17 clones that strongly recognized rDer f 1 were selected by ELISA. Five clones (1C9, 1G8, 3D7, 13C5, and 13F7) that showed good reactivity both in ELISA and western blotting were selected for further cloning (**Figure A and B**). However, western blotting showed that clones 1C9 and 13F7 did not retain the ability to recognize Der f 1. The combination of 3D7 as a capture antibody and biotinylated 13F7 as a detection antibody showed optimal results for the detection of Der f 1 in the extract. The detection limits were as low as 50 ng/mL of extract and 2 pg/mL of rDer f 1 (**Figure C and D**).

Der f 1 content in the 4 commercial extracts was 3.47–19.16 μg/mL when using the Indoor Biotechnologies antibodies and 2.79–18.59 μg/mL with the investigatory antibodies (**Supplementary Fig. S1**). No significant difference was shown between the results obtained by the 2 kits ($P$ = 0.1 to 0.7). Der f 1 concentrations in the 21 dust samples were 18–363.2 ng/g dust with Indoor Biotechnologies antibodies and 14–322.5 ng/g dust with the investigatory

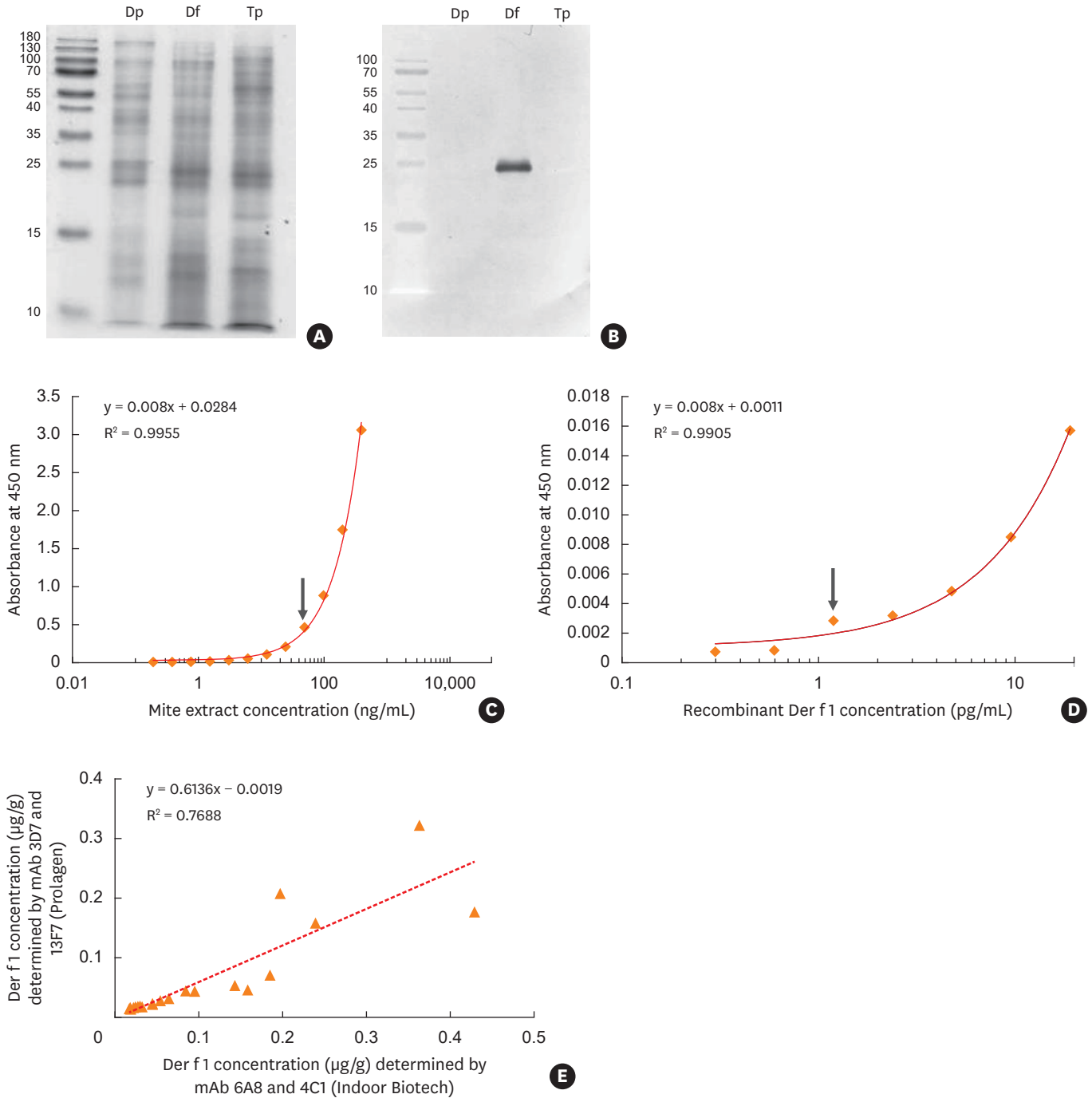


**Figure.** Specificity of monoclonal antibodies to Der f 1. Analysis by 15% sodium dodecyl sulfate polyacrylamide gel electrophoresis of allergen extracts from three species of dust mite: *Dermatophagoides pteronyssinus* (Dp), *D. farinae* (Df), and *Tyrophagus putrescentiae* (Tp) (A). Detection of native Der f 1 by monoclonal antibodies 3D7 (B). Detection of native Der f 1 in allergen extract by a two-site ELISA system. The two-site ELISA was quantified with doubling dilutions of the extract from 390 ng/mL of the extract (C). The two-site ELISA was quantified with doubling dilutions from 20 pg/mL of recombinant Der f 1 (D). Arrows indicate the detection limits. Comparison of Der f 1 concentration in house dust samples quantified by two ELISA systems (E).
mAb, monoclonal antibody; ELISA, enzyme-linked immunosorbent assay.

Jie Eun Yuk
https://orcid.org/0000-0003-2517-2653
Hangyeol Song
https://orcid.org/0000-0003-0139-733X

antibodies (**Figure E**). The Pearson's correlation coefficient between the 2 assays was 0.876824 ($P < 0.0001$). As expected, Der f 1 content in the commercial extracts and dust samples was not significantly different between the two-site ELISA systems.


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Hyunho Kim
https://orcid.org/0000-0001-7042-6566
Sung Hyun Kim
https://orcid.org/0000-0002-6498-7946
Dong Jun Kim
https://orcid.org/0000-0002-3666-0428
Yeji Shin
https://orcid.org/0000-0002-4145-5891
Deug-Chan Lee
https://orcid.org/0000-0002-4577-6318
Kyoung Yong Jeong
https://orcid.org/0000-0001-9887-1426
Jung-Won Park
https://orcid.org/0000-0003-0249-8749

**Disclosure**

JD Kim, KY Jeong, KH Park, JH Lee, and JW Park share equivalents of Prolagen, Ltd. JW Park reports serving as an unpaid chief technology officer for Prolagen. KY Jeong is a Technical Advisor of Prolagen. JT Kim and JD Kim are co-chief executive officers. HH Kim, SH Kim, DJ Kim, and YJ Shin are employees of Prolagen. The interests of these authors did not influence academic fairness in conducting this study, analyzing results, and writing a paper. Other authors have no potential conflicts of interest to disclose.

In conclusion, we developed novel mAbs against rDer f 1 useful for both qualitative and quantitative analyses. The antibodies should also be useful for the assessment of allergen exposure.

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## SUPPLEMENTARY MATERIAL

### Supplementary Fig. S1

Comparison of Der f 1 content in commercial *Dermatophagoides farinae* extracts quantified by two enzyme-linked immunosorbent assay systems.

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