An enzyme-linked immunosorbent assay for the detection of antigen-specific rat immunoglobulin E with improved sensitivity upon a conventional horseradish peroxidase-based ELISA method

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Animal models are currently used for screening the allergenic potential of foods and food products. However, there is a need to further develop the use of animal models so as to better identify the reactive sequences or specific allergens contained within the proteins recognised by serum IgE. Investigation of the epitope specificity of such IgE responses however, require high levels of IgE or a highly sensitive assay method. Current ELISA techniques frequently employ HRP as the tracer enzyme, which is not the enzyme most suited to the task of evaluation of low-level analytes. An AChE-based ELISA has been developed to detect β-lactoglobulin-specific rat IgE, adapted from a recently published method for the detection of specific human IgE [1]. As a result of higher catalytic turnover and absence of product inhibition with improved sensitivity for PLG-specific human IgE. Optical densities were measured at 450nm and 410nm respectively (samples assayed in duplicate).

Figure 1. Reproducibility of the AChE-based βLG-specific rat IgE ELISA, based upon the assay of 10 serum samples (8 replicates). OD measured at 410nm. Serum dilution = 1/10.

Figure 2. Comparison of the sensitivity of HRP-(O) and AChE-based βLG-specific rat IgE ELISAs. Optical densities were measured at 450nm and 410nm respectively (samples assayed in duplicate).

Figure 3. βLG-specific rat IgE profile in response to a milk immunisation regimen (as measured with AChE ELISA). Group of 5 male Brown Norway rats were exposed ip. to 500μg SSM (plus 1mg CGN) on days 0 and 7, followed by 100μg βLG (B variant) (plus 1mg CGN) on days 14 and 28. (P < 0.05)

These studies have utilised a modification of an AChE-based ELISA for βLG-specific human IgE to that of rat IgE. They demonstrate the increased sensitivity of the assay over a conventional HRP-based ELISA system [2]. The improved facets will allow the elucidation of more subtle responses, as well as aiding investigation into epitope recognition of antibodies.

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2. Matthews, G.S., Wal, J-M., Creminon, C. and Miller, K. (manuscript submitted)