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"***Amazing Biochemistry"***

Development and comparison of Western blot, dot blot and ELISA for mussel tropomyosin quantification

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Tropomyosin (TPM) is considered a major allergen among different shellfish species. Developing sensitive, specific, and reliable methods for quantifying TPM in food products is crucial for persons allergic to shellfish. We have previously developed a highly sensitive sandwich ELISA method for quantifying shrimp TPM. Despite high amino acid sequence homology between shrimp and mussels TPM, the method has not been reliable for quantifying TPM from mussels, underestimating its concentration up to three orders of magnitude. Therefore, this work aimed to develop alternative immunological methods for mussel TPM quantification. Western blot, dot blot, and indirect ELISA using monoclonal anti-TPM antibody and alkaline phosphatase-labeled secondary antibody were developed and compared in terms of their sensitivity. Tropomyosin in mussels extracts was quantified using highly purified natural shrimp tropomyosin as standard. The linear range for TPM quantification using dot blot was between 5 and 50 μg/mL, while Western blot has slightly increased sensitivity, with a linear range between 1.25 and 12.5 μg/mL. Indirect ELISA has further improved the sensitivity of TPM quantification, with a 0.04-0.4 μg/mL linear range. Additional work will be performed to enhance the sensitivity of the presented methods, with the final aim of reducing risks of inadvertent food contamination.

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