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One-step detection of ochratoxin A in cereal by dot immunoassay using a nanobody-alkaline phosphatase fusion protein

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Abstract

In this work, a polyvinylidene fluoride (PVDF) membrane-based dot immunoassay was developed using a nanobody-alkaline phosphatase (Nb-AP) fusion protein for one-step, qualitative, and visual detection of ochratoxin A in cereals. Using the optimal assay conditions, the assay was completed in 6 min and the cut-off limit of this method, assessed by the naked eye, was 5 μg/kg for ochratoxin A. This method was designed to be simple, fast, and low cost without time-consuming sample preparation and cleanup procedures. Good accuracy and reproducibility were obtained in a spike-and-recovery experiment. Sample analysis results of the Nb-AP-based dot immunoassay and LC-MS/MS were in good agreement with each other. These results demonstrated that the Nb-AP-based dot immunoassay could be an acceptable method for one-step and visual detection of ochratoxin A in cereal without the need for special instruments.

Keywords

ochratoxin A; one-step immunoassay; nanobody; alkaline phosphatase;