High-Throughput Enzyme Linked Immunosorbent Assay Performed by Molecule Immobilization on the Surface of Magnetic Particles

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Overview

This paper describes the detection of atrazine using enzyme linked immunosorbent assay (ELISA) by molecule immobilization on the surface of magnetic particles, and the separation of the particle bound complexes with the Thermo Scientific KingFisher Flex instrument, a magnetic particle processor. The ELISAs based on magnetic particle separation technique are faster to perform than the traditional ELISAs, which require more time-consuming separation steps of the unbound molecules.

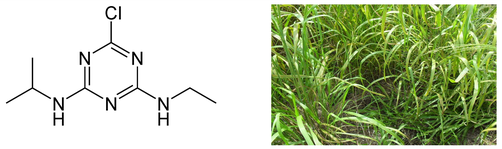
Introduction

Enzyme linked immunosorbent assays are used for the detection of specific antigens or antibodies in a sample. The assay is based on an immobilized antibody-antigen binding reaction in microplate format and includes several reagent addition, incubation and washing steps, therefore easily extending the total assay time to a couple of hours.

This work focused on the ELISA for detection of atrazine, performed by immobilizing the antibody-antigen complexes on the surface of magnetic particles, and separating the particle-bound complexes from the assay medium with a magnetic particle processor, the KingFisher® Flex.

Atrazine (Figure 1) is one of the most widely used herbicides around the world. It has been found to be less biodegradable than other less substituted triazine ring compounds. Because of its relative wide application, high persistence, and ability to leach through the soil, atrazine can be detected in rain, surface water and ground water. The use of atrazine is prohibited in several countries, but it is still very widely used in the US. Atrazine was banned in the European Union (EU) in 2004 because of its persistent groundwater contamination. Its endocrine effects, possible carcinogenic effect, and epidemiological connection to low sperm levels in men has led several researchers to call for banning it in the US.

FIGURE 1. The molecular structure of atrazine and some switchgrass treated with atrazine herbicide.

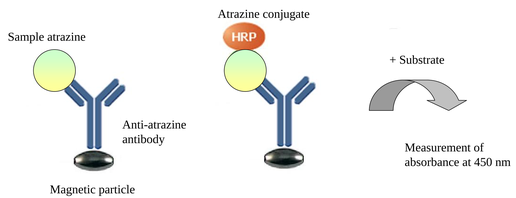


Methods

The principle of the magnetic particle atrazine ELISA is presented in figure 2.

The kit used for atrazine detection was Atrazine Magnetic Particle ELISA kit (PN 500001, Abraxis LLC, USA). The magnetic particle separation was performed on a Microtiter® deep well 96 plate (cat.no 95040450, Thermo Fisher Scientific), and the final colorimetric measurement was performed on a clear 96-well Immulon 1B microplate (cat.no 3355, Thermo Fisher Scientific)

FIGURE 2..The principle of the magnetic particle atrazine ELISA. The antibody coated magnetic particles bind both the sample atrazine and the atrazine conjugate (atrazine labeled with horseradish peroxidase, HRP) in a competitive assay.



The sample including the atrazine was first mixed in a non-coated microplate well with magnetic particles coated with an antibody against atrazine. The enzyme conjugated atrazine was added to the reaction to compete with the sample atrazine. After incubation the antibody-antigen-magnetic particle complexes were separated from the non-bound molecules by collecting them with the 96-well magnetic head of the KingFisher Flex (figure 4). The collected particles were then automatically moved by the magnet head to another plate for washing and finally eluted to the assay buffer on a new plate. The KingFisher Flex performed automatically all the separation, washing and elution steps of the protocol. The principle of the magnetic particle collection with KingFisher Flex is presented in figure 3.

FIGURE 3..The magnetic rod covered with a plastic tip comb collects the magnetic particles from the well. When the particles have bound to the magnet, they can be moved from one plate to another for washing and elution steps. The particles can be released from the magnetic head by lifting the magnet out of the tip comb and letting the particles to detach from the surface of the tip comb.

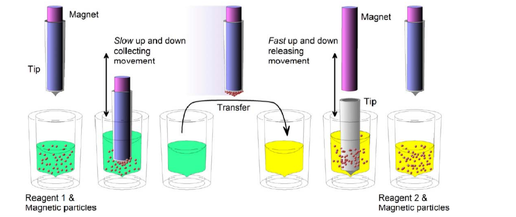


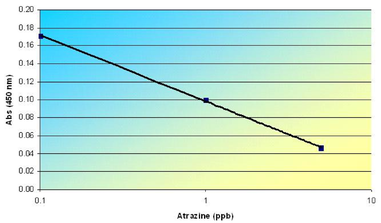
FIGURE 4. The magnetic head of the KingFisher Flex collects the magnetic particles from the wells of a deep well plate, and releases the particles to the wells of the next plate placed on the instrument turn table. The assay was performed on a 96 well plate, but these pictures illustrate the magnetic head movements on a 24 well plate.

Finally after the enzyme substrate addition, the absorbance of each sample was measured with a microplate photometer, Thermo Scientific Multiskan FC, controlled by the Thermo Scientific SkanIt Software. The absorbance intensity was inversely proportional to the atrazine concentration of the sample. The atrazine concentrations of the unknown samples were calculated with the in-built quantitative curve fit calculation of the SkanIt Software.

Results

The atrazine standard curve of the assay is presented in picture 5. Samples containing atrazine within the dynamic range (0.1-5.0 ppb) can be directly tested with this assay. In addition to atrazine, there are several other environmental analytes (e.g. glyphosate and estrogens) for which similar magnetic particle ELISAs are commercially available.

FIGURE 5. The atrazine standard curve of the assay measured with the Multiskan® FC photometer. The R_2= value of the curve was 0.9996.



Conclusions

The magnetic particle based ELISAs have many advantages over the traditional ELISAs. The optimized incubation times for binding reactions with magnetic particle separation speeded up the assay remarkably compared to traditional ELISAs, where the binding reaction happens by molecules immobilized on the bottom of the microplate well. In addition the washing procedure was faster, as the magnetic particle collection by the KingFisher Flex instrument replaced the need to wash away the unbound molecules with a separate microplate washer.

The KingFisher Flex is capable of separating the magnetic particles simultaneously from all the wells of a 96-well microplate by using the 96 well magnetic head. The instrument can be easily programmed to handle the whole assay protocol (separation, washing, elution) automatically. Using magnetic particles also eases the assay automation, as liquid transfer during the assay can be minimized. Therefore high-throughput ELISAs can be conveniently and reproducibly carried out with this kind of magnetic particle based molecule separation.

FIGURE 6. The KingFisher® Flex, a magnetic separation automate for 24- and 96-well microplates and the Multiskan® FC, a photometer for 96- and 384-well microplates.

Further Information

For further information about Thermo Scientific microplate instruments, please contact:

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