Time-Resolved Fluorometric Receptor Assay for the Measurement of IL-2-IL-2 Receptor α Interaction

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INTRODUCTION

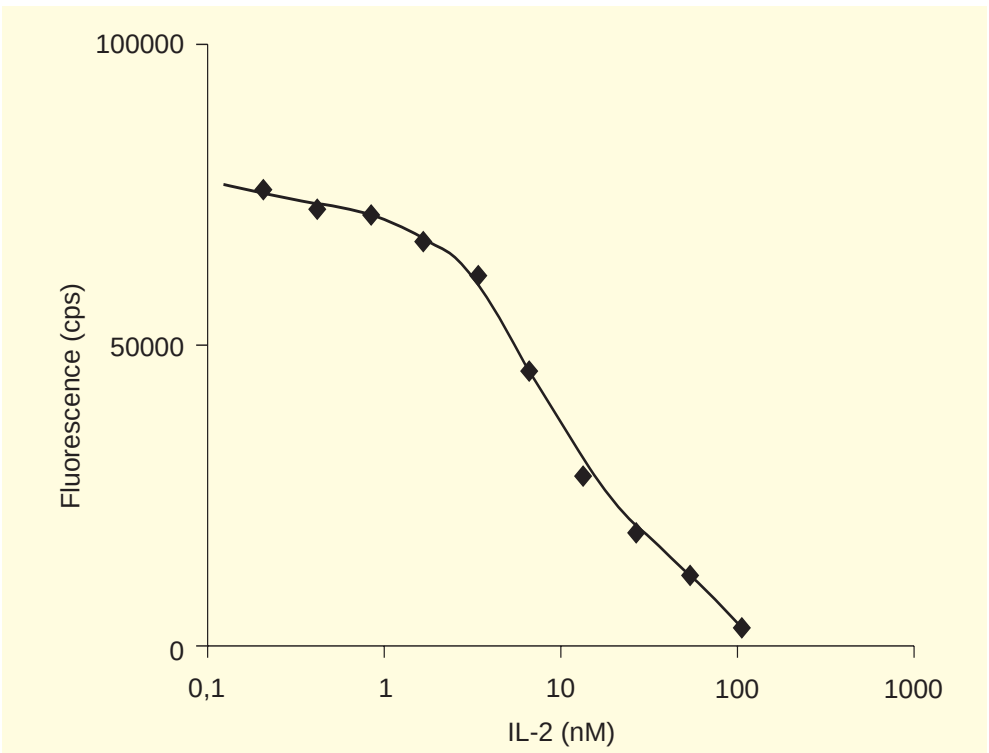
The lymphokine interleukin-2 (IL-2) plays a central role in the initiation of immunoreactions by stimulating proliferation and differentiaton of lymphocytes and other cells. We have developed an IL-2–IL-2 receptor α interaction assay relying on the highly sensitive fluorescence enhancement technique DELFIA® and coated microtitration plate wells. This assay format allows all manipulation steps to be automated.

ASSAY PRINCIPLE

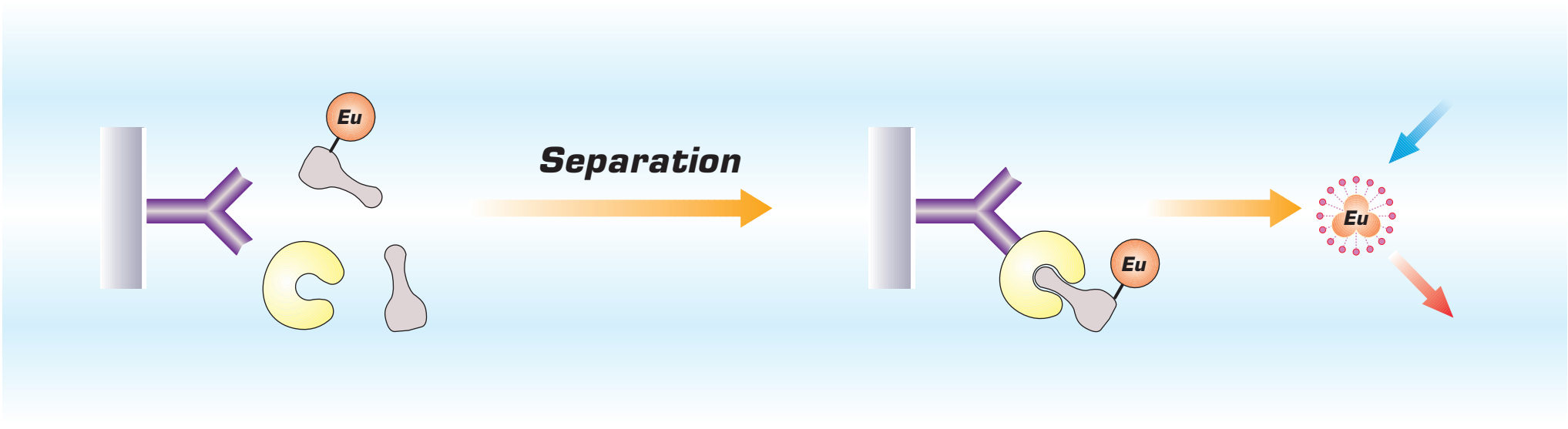
The assay consists of wells coated with monoclonal antibody to human IL-2 receptor α (IL-2Rα), europium-labeled IL-2 (Eu-IL-2) and human recombinant IL-2Rα subunits expressed using the baculovirus expression vector system. In the assay, IL-2Rα lysate was incubated in the MAb coated wells for 2 h at room temperature. The wells were washed before addition of Eu-IL-2 and incubated for 70 more minutes. After the incubation, unreacted Eu-IL-2 was separated from bound ligand by washing. Then DELFIA Enhancement Solution was added to the wells. Receptor-bound Eu dissociates into the enhancement solution where it forms highly fluorescent complexes. The fluorescence was measured in the Wallac 1420 VICTOR™ multilabel counter.

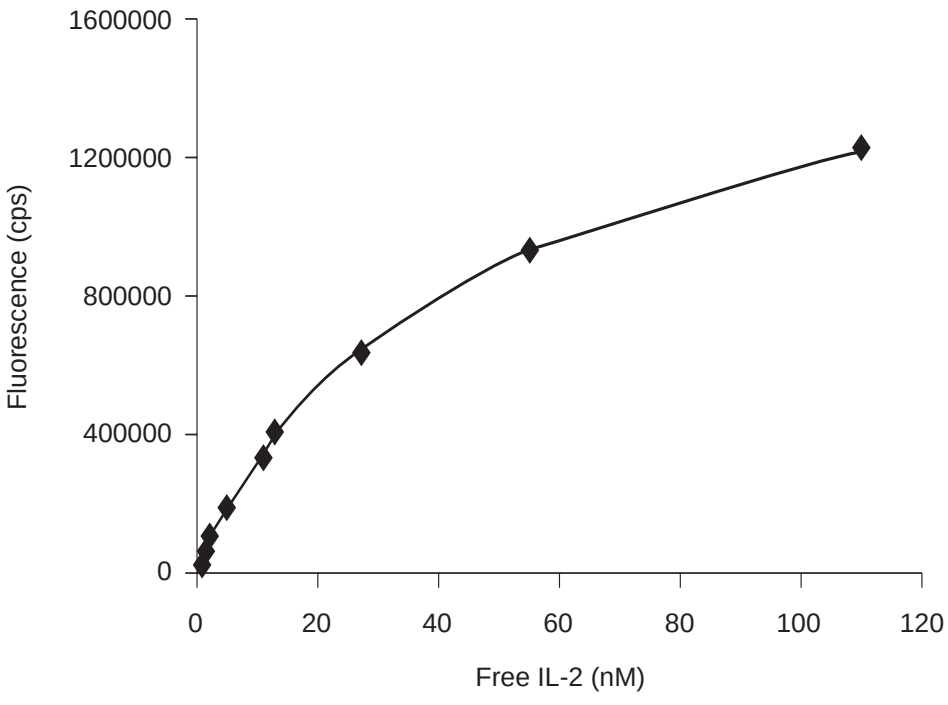
RESULTS

Saturation curve



The Kd value calculated from the saturation curve was 2.6×10^{-8} M.





Displacment curve and precision

The displacement curve gave a 50% inhibition with an IL-2 concentration of 0.9×10^{-8} M. The pooled CV% for triplicate measurements in the assay was 5%.

CONCLUSIONS

This receptor assay format provides convenient separation of bound ligand from unbound and is therefore easy to automate and suitable for high throughput screening applications.

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