

Drug Discovery | Research | Clinical Screening

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DELFIA®

HIGH THROUGHPUT SCREENING

Multiplexing DELFIA® assays using lanthanide-labeled probes

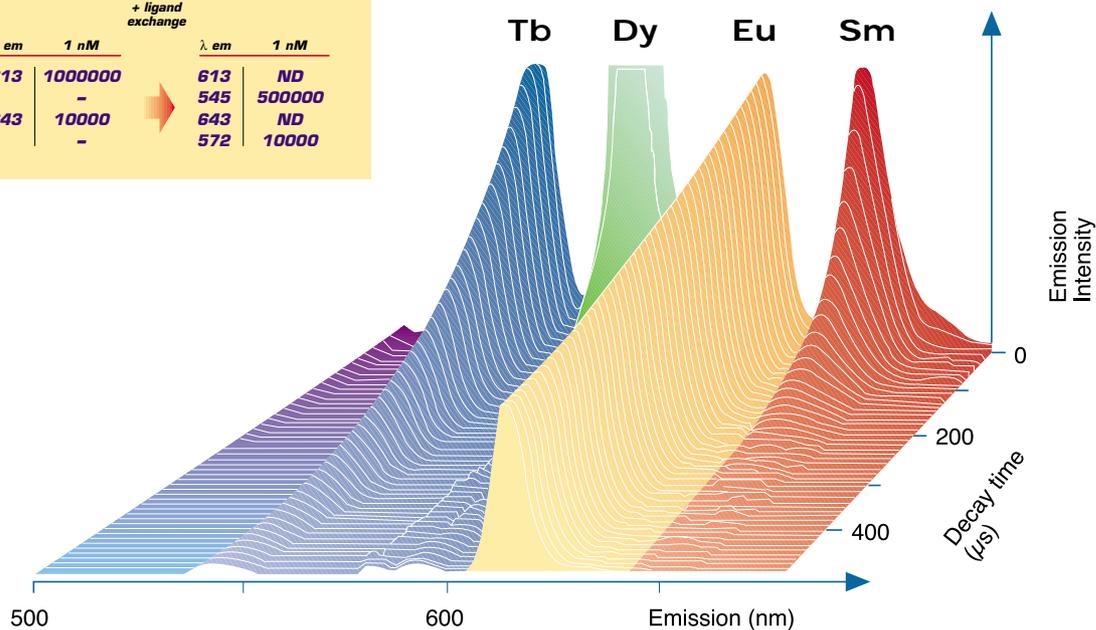
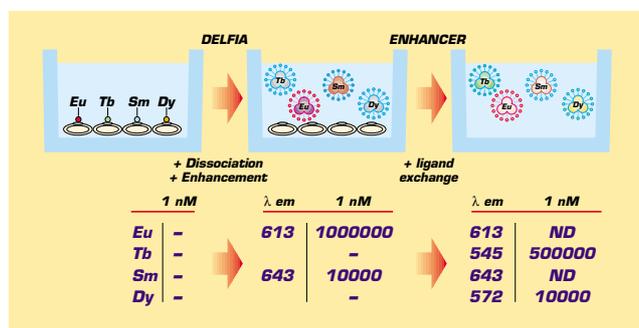


Principle and applications

Starting from theoretical ideas about their potential application as labels in the early 1970s, through the development of the first working system – dissociation enhanced lanthanide fluoroimmunoassay (DELFA) – in the early 1980s, lanthanides have found widespread application, particularly in neonatal and prenatal screening where both immunological and hybridization recognition reactions are utilized. Today, time-resolved fluorometry (TRF) has attracted great interest as a tool for application in a range of assay formats in drug screening.

Lanthanide chelates have unique fluorescence properties that serve them well as sensitive labels. For example, they have long fluorescence decay after excitation so it is possible to detect fluorescence signals even after a long time delay, which virtually eliminates all background fluorescence. Lanthanide chelates also display a large Stoke's Shift compared to traditional labels. Such a large Stoke's Shift minimizes crosstalk between excitation and emission signals and contributes to a high signal-to-noise ratio. Moreover lanthanides are suitable for quantitative multianalyte assays because of their narrow emission peaks at different wavelengths and their different fluorescence lifetime. The combination of spectral window and time window can be utilized for the optimization of the measurement parameters in order to obtain maximal sensitivity and to minimize signal spillover.

Labelling with Europium (Eu) or Samarium (Sm) chelates follows the same general protocol. Therefore, experience gained with labelling proteins, nucleotides etc. with Eu-chelate is directly useful when Sm- and Terbium (Tb)-labels are used for multiplexing assays. Eu gives high fluorescence and has the best sensitivity. Sm or Tb label should be used as the second label in dual-label assays for measuring the analyte requiring the lower sensitivity. 200 µl of Enhancement Solution is recommended (using a 96-well plate) to develop the signal for both Eu and Sm. For Tb label, an additional 50 µl of Enhancer is needed.



Chelate	Ex. (nm)	ε (L/M cm)	Em. (nm)	τ (µs)	Q
DELFA					
Eu-2-NTA3	340	36,000	615	730	0.69
Sm-2-NTA3	340	36,000	642	50	0.02
Tb-TMP-DPA	320	18,800	545	1050	0.48
Dy-TMP-DPA	320	18,800	572	16	0.02

Selected reagents and consumables for multiplexing assay

1244-360	DELFLIA Eu-labelled streptavidin, 250 µg
1244-361	DELFLIA Eu-labelled protein G, 250 µg
1244-330	DELFLIA Eu-labelled anti-human IgG, 100 µg
AD0105	DELFLIA Eu-labelled anti-rabbit IgG, 200 µg
AD0124	DELFLIA Eu-labelled anti-mouse IgG, 50 µg
AD0049	DELFLIA Sm-labelled streptavidin, 50 µg
AD0047	DELFLIA Tb-labelled streptavidin, 50 µg
1244-114	DELFLIA Wash Concentrate, 250 mL
1244-106	DELFLIA Assay Buffer, 50 mL
1244-104	DELFLIA Enhancement Solution, 50 mL
C500-100	DELFLIA Enhancer, 50 mL
B119-100	DELFLIA 1 nmol/L Europium standard solution, 50 mL
B115-100	DELFLIA 10 nmol/L Samarium standard solution, 50 mL
C558-100	DELFLIA 1 nmol/L Terbium standard solution, 50 mL
1244-302	DELFLIA Eu-labelling kit, up to 1 mg of protein
1244-303	DELFLIA Sm-labelling kit, up to 1 mg of protein
AD0009	DELFLIA Tb-N1 ITC chelate and Tb standard, 1 mg
AAAND-0001	DELFLIA yellow plate, pack of 60
AAAND-0003	DELFLIA anti-mouse coated yellow plate, pack of 10
AAAND-0004	DELFLIA anti-rabbit coated yellow plate, pack of 10
CC33-1210	DELFLIA anti-sheep coated yellow plate, pack of 10
AAAND-0005	DELFLIA streptavidin coated yellow plate, pack of 10

For custom labeling and assay development needs please contact your local sales representative, or e-mail us at assayservices@perkinelmer.com

Selected recent publications about multiplexing assays using lanthanide-labeled probes

A fast and robust dual-label nonradioactive oligonucleotide ligation assay for detection of factor V Leiden.

Chakravarty A, Hansen TS, Hørder M, Kristensen SR

Thromb Haemost 1997 Oct 78:1234-6

Abstract

Activated protein C resistance is in almost all cases caused by the factor V Leiden mutation (FV:R506Q). Due to the high prevalence and clinical significance of the mutation reliable methods suited for processing large sets of samples are in demand. We here present the oligonucleotide ligation assay (OLA) with lanthanide labeled oligonucleotides for the detection of FV Leiden. The assay is based on time resolved fluorescence measurement of lanthanide labeled oligonucleotides (DELFLIA: Delayed Enhanced Lanthanide Fluorescence Immuno Assay) and on the specificity of T-4 DNA Ligase to join two adjacent oligonucleotides only when the two are complementary to the PCR template at the ligation junction. The Europium/Samarium fluorescence pattern is specific for each of the three genotypes (G/G, G/A, A/A) and clearly separates the three genotypes. By using a wildtype probe (Samarium labeled) and a mutant-specific probe (Europium labeled) simultaneously an internal control of the assay is included in each reaction. The assay is simple to perform, can be partly automated and is ideal for processing large sets of samples.

Differentiation of cytotoxicity using target cells labelled with europium and samarium by electroporation.

Bohlen H, Manzke O, Engert A, Hertel M, Hippler-Altenburg R, Diehl V, Tesch H

J Immunol Methods 1994 Jul 173:55-62

Abstract

We report the simultaneous use of europium-DTPA (Eu-DTPA) and samarium-DTPA (Sm-DTPA) in cytotoxicity experiments to analyze simultaneously LAK and NK cell lysis and to differentiate between specific target lysis and bystander killing. The target cells were either labelled with Eu-DTPA or Sm-DTPA chelates by electroporation, which permits the use of target cell lines or primary leukemic B cells (B-CLL) that cannot be labelled by the conventional dextran-sulphate method. The release of europium and samarium reaches a maximum at comparable time intervals (2-3 h). Due to the shorter counting interval within the samarium window the labelling efficiency is about ten times less efficient compared to europium. Using europium as label for the LAK target Daudi and samarium as label for the NK sensitive cell line K562 the differentiation of LAK versus NK activity can be performed in a single culture assay. Also, the killing of B cells and bystander cells by cytotoxic T cells was analyzed in a system where T cells were redirected to B cells through CD3 x CD19 bispecific antibodies. In fact, no bystander killing was noted when bispecific antibodies were used to bridge cytotoxic T cells to the B cells. This approach provides a simple non-radioactive method for evaluating cytotoxicity against two different cells in a single culture well.

Dual-label time-resolved fluoroimmunoassay of psychopharmaceuticals and stimulants in serum.

Kimura H, Mukaida M, Wang G, Yuan J, Matsumoto K

Forensic Sci Int 2000 Sep 113:345-51

Abstract

A new method to measure two different drugs simultaneously by time-resolved fluoroimmunoassay (TR-FIA) has been developed. In the TR-FIA reported here, psychopharmaceuticals [chlorpromazine (CPZ) and desipramine (DSP)] and methamphetamine (MA) contained in serum are assayed by a combined use of a new europium (Eu) chelate and a samarium (Sm) chelate, as labels. The drug concentrations were determined by the competition between a labeled antigen with Eu(3+) or biotin and a sample antigen. A microtiter plate coated with a mixture of rabbit IgGs (anti-MA and anti-CPZ or anti-MA and anti-DSP) was used. In the assay of MA and CPZ, Eu(3+) labeled MA-bovine serum albumin conjugate (MA-BSA) and biotinylated CPZ-BSA were added to the well with their non-labeled standard solutions or samples. MA was assayed by measuring the fluorescence intensity of Eu(3+) at 615 nm. After incubation of the Sm(3+) labeled streptavidin, CPZ was assayed by measuring the fluorescence of Sm(3+) at 643 nm. In the assay of MA and DSP, Eu(3+) labeled DSP-BSA and biotinylated MA-BSA were used. In our dual-assay, the minimum detection limits of these drugs were 1ng/ml for MA, 10 ng/ml for CPZ and 10 ng/ml for DSP. Since the simultaneous detection of different drugs by TR-FIA is time and sample saving, the method can be employed in rapid and sensitive screening tests.

Development of a dual-label time-resolved fluorometric immunoassay for the simultaneous detection of two recombinant proteins in potato.

Bookout JT, Joaquim TR, Magin KM, Rogan GJ, Lirette RP

J Agric Food Chem 2000 Dec 48:5868-73

Abstract

Immunological methods such as ELISA have been traditionally employed to quantify protein levels in plants improved through modern biotechnology. Combined trait products (i.e., plants producing multiple recombinant proteins) created by introducing multiple genetic traits by transformation or traditional breeding methods have prompted the need for the development of analytical assay technologies capable of detecting and quantifying multiple proteins in a single assay. The development of a two-site, sandwich, dual-label, time-resolved fluorometry-based immunoassay (TRFIA) capable of simultaneously quantitating two recombinant proteins (CP4 EPSPS and Cry3A) in plant sample extracts of genetically improved potato cultivars is reported here. The performance characteristics of TRFIA were similar to or exceeded those of current ELISA methods used to detect and quantitate these proteins. TRFIA is a practical and reliable assay for the quantitation of proteins in genetically improved potato plants and offers an alternative approach to conventional ELISA methods with the added benefit of multiple analyte detection.

Simultaneous quantitation of diphtheria and tetanus antibodies by double antigen, time-resolved fluorescence immunoassay.

Aggerbeck H, Nørgaard-Pedersen B, Heron I

J Immunol Methods 1996 Apr 190:171-83

Abstract

A dual, double antigen, time-resolved fluorescence immunoassay (DELFI) for the simultaneous detection and quantitation of diphtheria (D) and tetanus (T) antibodies in sera has been developed. In the double antigen format one arm of the antibody binds to antigen coated microtitre wells and the other arm binds to labelled antigen to provide a fluorescent signal. This assay was found to be functionally specific for IgG antibodies and showed a good correlation with established toxin neutralization assays. Furthermore, the double antigen set-up was species independent, permitting the direct use of existing international references of animal origin to measure protective antibody levels in humans in international units (IU/ml). The detection limit corresponded to 0.0003 IU/ml with Eu(3+)-labelled toxoids and to 0.0035 IU/ml using Sm(3+)-labelled toxoids. The assay was fast with a high capacity making it a suitable method for serological surveillance studies.

Seven-color time-resolved fluorescence hybridization analysis of human papilloma virus types.

Samiotaki M, Kwiatkowski M, Ylitalo N, Landegren U

Anal Biochem 1997 Nov 253:156-61

Abstract

Identification of human papilloma virus (HPV) types is important in order to determine the risk of cervical carcinoma in women. This requires a technique to probe individual samples for multiple virus specificities. Here we describe simultaneous multicolor analysis of amplification products for any of seven amplified HPV types 16, 18, 31, 33, 35, 39, and 45, associated with cancer of the cervix. A seminested polymerase chain reaction was performed in a single tube using a biotinylated inner primer. Sets of amplification products, immobilized on a 96-pronged manifold solid support, were rendered single stranded and probed with a mix of seven type-specific, differentially labeled oligonucleotides. These probes contained 10 or 20 lanthanide chelates at the 5' ends with seven distinct combinations of europium, terbium, and samarium ions. The seven viral strains were correctly identified by time-resolved fluorescence measurement of the specifically hybridized probes. Using this assay format, simultaneous detection of any of seven or even more target variants is possible.

Simultaneous detection of IFN-gamma and IL-4 mRNAs using RT-PCR and time-resolved fluorometry.

Halminen M, Sjöroos M, Mäkelä MJ, Waris M, Terho E, Lövgren T, Ilonen J

Cytokine 1999 Jan 11:87-93

Abstract

Time-resolved fluorometry was applied in the detection of RT-PCR amplified mRNAs for the Th1 and Th2 cell-derived cytokines interferon gamma (IFN-gamma) and interleukin (IL-)4, respectively. RNA stimulated cells was reverse transcribed and the cDNAs for the cytokine mRNAs and the constantly expressed beta-actin (beta-ACT) mRNA were simultaneously amplified in one multiplex PCR reaction. The PCR conditions were optimized to minimize mutual inhibition of individual amplifications. One of the PCR primers in each primer pair was biotinylated, and the PCR products were captured onto streptavidin-coated microtitre plates. The three PCR products were detected with three different lanthanide labelled target-specific probes in solution hybridization. IFN-gamma, IL-4 and beta-ACT were detected with europium (Eu), terbium (Tb) and samarium (Sm) labelled probes, respectively, using time-resolved fluorometry. Small cell numbers used in microtitre plate cultures were sufficient to detect cytokine messages after mitogen stimulation. This sequence-based method provides a sensitive, specific, fast and nonisotopic alternative to conventional blotting and hybridisation with radioactive probes. In addition, the multiplex fluorogenic dye detection facilitates relative quantification of target mRNAs.

Simultaneous determination of alpha-fetoprotein, human chorionic gonadotropin and estriol in serum of pregnant women by time-resolved fluoroimmunoassay.

Ito K, Oda M, Tsuji A, Maeda M

J Pharm Biomed Anal 1999 Jun 20:169-78

Abstract

We have developed a simple and rapid time-resolved fluoroimmunoassay (TR-FIA) for simultaneous determination of alpha-fetoprotein (AFP), human chorionic gonadotropin (hCG) and estriol (E3) using europium and samarium ion chelate. In the proposed method, we used a combination of a 96-well microtiter plate for the AFP and hCG assay and transferable solid phase plate for the E3 assay. Therefore, these analytes could be measured simultaneously. The measurable ranges for AFP, hCG and E3 by the proposed method were 3.91-1000 ng ml(-1), 877-250000 IU l(-1) and 0.39 100 ng ml(-1), respectively. The proposed method which utilized characteristics of a rare earth ion chelate, was convenient (unnecessary diluting samples), quick (96 assays for 2 h), and required only a small quantity sample (50 microl). The principle of this proposed method is applicable to other antigens.

Peptide antagonists of the human estrogen receptor.

Norris JD, Paige LA, Christensen DJ, Chang CY, Huacani MR, Fan D, Hamilton PT, Fowlkes DM, McDonnell DP

Science 1999 Jul 285:744-6

Abstract

Estrogen receptor alpha transcriptional activity is regulated by distinct conformational states that are the result of ligand binding. Phage display was used to identify peptides that interact specifically with either estradiol- or tamoxifen-activated estrogen receptor alpha. When these peptides were coexpressed with estrogen receptor alpha in cells, they functioned as ligand-specific antagonists, indicating that estradiol-agonist and tamoxifen-partial agonist activities do not occur by the same mechanism. The ability to regulate estrogen receptor alpha transcriptional activity by targeting sites outside of the ligand-binding pocket has implications for the development of estrogen receptor alpha antagonists for the treatment of tamoxifen-refractory breast cancers.

Merged Screening for Human Immunodeficiency Virus Tat and Rev Inhibitors.

Hamy F, Felder E, Lipson K, Klimkait T

Journal of Biomolecular Screening, Volume 6, Number 3, 2001

Abstract

In addition to "conventional" drug discovery targets used in modern strategies, mainly focusing on proteins, recent insights into gene regulation as a novel drug concept have begun to invite the targeting of biomolecular interactions between proteins and RNA. Because two protein-RNA interactions (Tat and trans-activation-responsive element, Rev and Rev-responsive element) are essential for any productive replication of human immunodeficiency virus, this important human pathogen was used as a model system for our studies. The design of a fluorescence-based high throughput assay, in which both targets were presented in the same vessel, enabled us to simultaneously interrogate two characteristics of a potential inhibitor: potency of interference and selectivity toward each of the interactions. Although related systems have been reported for several DNA binders, an extension into interference with transcription events would open a new dimension of cellular regulation. Here we describe the setup of the screening assay for over 110,000 compounds as well as a primary characterization of identified hits. The assay's characteristics demonstrate that a microwell-based dual screening system for RNA binders may add a powerful tool to modern drug discovery.

Diagnosis of Enterovirus and Rhinovirus Infections by RT-PCR and Time-Resolved Fluorometry with Lanthanide Chelate Labeled Probes.

Lönnrot M, Sjöroos M, Salminen K, Maaronen M, Hyypiä T, Hyöty H,
Journal of Medical Virology 59: 378-384 (1999)

Abstract

Detection of enteroviruses and rhinoviruses has traditionally been based on laborious and time-consuming virus isolation. Recently, rapid and sensitive assays for detecting enterovirus and rhinovirus genomic sequences by reverse transcription-polymerase chain reaction (RT-PCR) have been introduced. An RT-PCR assay is described that amplifies both enteroviral and rhinoviral sequences, followed by liquid-phase hybridization carried out in a microtiter plate format. In the hybridization assay, amplicons are identified by enterovirus- or rhinovirus-specific probes carrying lanthanide chelate labels, which can be detected simultaneously by time-resolved fluorometry. The sensitivity and specificity of the RT-PCR-hybridization method were evaluated with a representative collection of enteroviruses and rhinoviruses and tested further its applicability to the clinical setting with cerebrospinal fluid samples and nasopharyngeal aspirates. The RT-PCR assay amplified all enteroviruses and rhinoviruses tested, and all but one amplicon gave a positive result in the subsequent hybridization assay. The RT-PCR-hybridization method was more sensitive than virus isolation for the detection of enteroviruses and rhinoviruses in the clinical samples. High sensitivity, rapidity, and easy performance make the assay suitable for the routine diagnosis of enterovirus and rhinovirus infections.

Measurement of the Complex between Prostate-specific Antigen and (1-Protease Inhibitor in Serum.

Zhang W-M, Finne P, Leinonen J, Vesalainen S, Nordling S, Stenman U-H
Clinical Chemistry 45:6, 814-821 (1999)

Abstract

Background: Prostate-specific antigen (PSA) occurs in serum both free and in complex with protease inhibitors. The complex with (1-antichymotrypsin (ACT) is the major form in serum, and the proportion of PSA-ACT is higher in prostate cancer (PCa) than in benign prostatic hyperplasia (BPH). PSA also forms a complex with (1-protease inhibitor (API) in vitro, and the PSA-ACT complex has been detected in serum from patients with prostate cancer. The aim of the present study was to develop a quantitative method for the determination of PSA-API and to determine the serum concentrations in patients with PCa and BPH.

Methods: The assay for PSA-API utilized a monoclonal antibody to PSA as capture and a polyclonal antibody to API labeled with a Eu-chelate as a tracer. For calibrators, PSA-API formed in vitro was used. Serum samples were obtained before treatment from 82 patients with PCa, from 66 patients with BPH, and from 22 healthy females. **Results:** The concentrations of PSA-API are proportional to the concentrations of total PSA. PSA-API comprises 1.0-7.9% (median, 2.4%) of total immunoreactive PSA in PCa and 1.3-12.2% (median, 3.6%) in BPH patients with serum PSA concentrations > 4 µg/L. In patients with 4-20 µg/L total PSA, the proportion of PSA-API serum is significantly higher in BPH (Median, 4.1%) than in PCa (median, 3.2%; P = 0.02).

Conclusions: The proportion of PSA-API in serum is lower in patients with PCa than in those with BPH. These results suggest that PSA-API is a potential adjunct to total and free PSA in the diagnosis of prostate cancer.

A competitive dual-label time-resolved immunofluorometric assay for simultaneous detection of carbonic anhydrase I and II in cerebrospinal fluid.

Parkkila A-K, Parkkila S, Serlo W, Reunanen M, Vierjoki T, Rajaniemi H
Clinica Chimica Acta 230: 81-89 (1994)

Abstract

Carbonic anhydrase (CA) is functionally an important enzyme in the central nervous system (CNS) where it is involved in the control of acid-base balance and regulation of the production of cerebrospinal fluid (CSF). Isoenzyme II (CAII) is the most widely distributed CA in the CNS being specifically present in CNS glial tissue and therefore it is expected to be leaked to CSF in degenerative CNS diseases. A competitive dual-labeled time-resolved immunofluorometric assay was developed for simultaneous quantification of human CAI (HCA I) and II (HCA II) in CSF. HCA I was measured to determine the blood contamination in the samples. This solid-phase immunoassay is based on competition between europium (Eu³⁺)- or samarium (Sm³⁺)-labeled antigen and the sample antigens for polyclonal rabbit antibodies which are attached to microtiter-plate wells precoated with sheep anti-rabbit IgG. The subsequent immunoassay, including the separation of free and bound HCA I and II, requires only one incubation step, after which an enhancement solution dissociates Sm³⁺ and Eu³⁺ ions from the labeled HCA I and II, respectively, into a solution where they form highly fluorescent chelates. Spectra of the fluorescent chelates in the microtitration strip wells were run on time-resolved fluorometers equipped with filters for Eu³⁺ (613 nm) and Sm³⁺ (643nm), the fluorescence from each sample being inversely proportional to the concentration of antigens. The detection limit of the HCA II assay was 0.3 µg/L and that of the HCA I assay was 5.2 µg/l. The intra- and inter-assay imprecisions (C.V.s) were 8.0% and 8.8% for HCA I and 6.3% and 4.8% for HCA II, respectively. The analytical recovery ranged from 96 to 110% for HCA I and from 95 to 108% for HCA II. The concentration of HCA II derived from brain tissue present in the CSF of hydrocephalic children varied between 1.0 and 35.9 µg/l (n = 25).

Triple-Label Hybridization Assay for Type-1 Diabetes-Related HLA alleles.

Sjöroos M, Iitiä A, Ilonen J, Reijonen H, Lövgren T

BioTechniques Vol. 18, No.5: 870-877 (1995)

Abstract

We describe a method for the detection of two type 1 (insulin-dependent) diabetes susceptibility (*0201, 0302) alleles and two protective (*0301, *0602/0603) alleles of the HLA-DQB1 gene on the human major histocompatibility complex (MHC). The test is based on DNA amplification with PCR followed by simultaneous, allele-specific triple-label hybridization performed in microtitration wells. In the hybridization, very short allele-specific oligonucleotides labeled with europium (Eu), terbium (Tb) or samarium (Sm) are used. The labeled probes could be detected using time-resolved fluorometry with sensitivities of 1×10^7 , 3×10^8 and 3×10^8 molecules, respectively. Cross-reactions were not found among samples containing 14 common DQB1 alleles. To test the utility of the developed assay, 100 DNA and 14 dried blood spot samples with known DQB1 alleles were analyzed. A 100% agreement with the reference assay method was reached. Thus, this triple-label hybridization assay proved to be suitable even for detection of a large number of samples.

Simultaneous measurement of natural killer cell cytotoxicity against each of three different target cell lines.

Blomberg K

Journal of immunological methods 168: 267-273 (1994)

Abstract

A time-resolved fluorometric assay for the simultaneous measurement of natural killer cell activity against three different lanthanide diethylenetriaminopentaacetate (LaDTPA) labelled target cell lines is described. The target cell line K-562 was labelled with SmDTPA, the cell line Molt with TbDTPA and the cell line Raji with EuDTPA. After co-incubation of the three target cell lines with effector cells the fluorescence of the lanthanides released from the lysed target cells was measured in an enhancer solution in which they formed highly fluorescent complexes. It was possible to differentiate the specific release from the three target cell lines because the emission lines of the europium, samarium and terbium complexes formed in the enhancer solution are well separated from each other. The autofluorescence from culture media supplemented with serum was avoided by the use of time-resolved fluorometry. The results show that applying fluorometry based on the combination of spectral and temporal resolution to natural killer cell assays, makes possible the simultaneous determination of lysis in up to three target cell lines in complex culture medium.



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