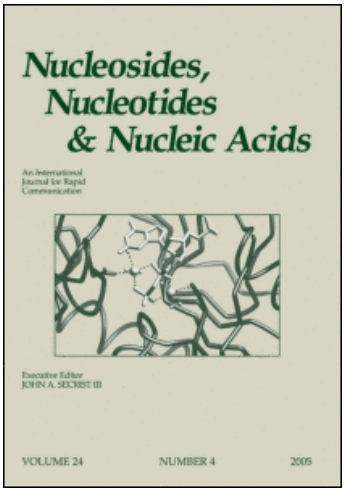

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New Homogeneous Reverse Transcriptase and Nuclease Assays Based on Rare Earth Cryptate and Fluorescent Energy Transfer

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NEW HOMOGENEOUS REVERSE TRANSCRIPTASE AND NUCLEASE ASSAYS BASED ON RARE EARTH CRYPTATE AND FLUORESCENT ENERGY TRANSFER

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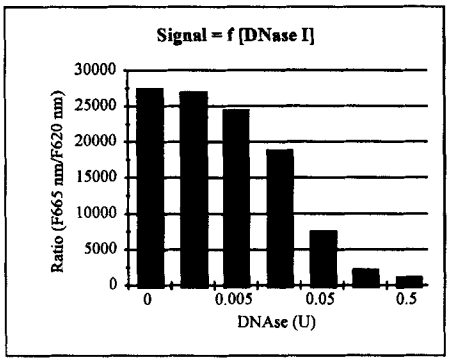
ABSTRACT: An homogeneous non isotopic technique based on time-resolved fluorescence, europium trisbipyridine cryptate ($TBPEu^{3+}$) as fluorescent donor and non radiative energy transfer to an acceptor (cross-linked allophycocyanine, XL665) has been used to develop new assays. These formats provide rapid and straightforward measurement of reverse transcriptase (RTase) and DNase activity and for example a mean to screen for RTase inhibitors.

The Homogeneous assay [1,2]: Our Time-Resolved Fluorescence technique is based on the amplification of the long-lived fluorescence of $TBPEu^{3+}$ donor by a Förster non radiative energy transfer on XL665 acceptor. The time resolved measurement allows a clear distinction between the long-lived emission of XL665 engaged in the energy transfer and its short natural fluorescence as free molecule. The spectral selectivity (620 nm for $TBPEu^{3+}$, 665 nm for XL665) allows to get rid of the media transmission by measuring the ratio of fluorescence : ($R = F_{665nm} / F_{620nm}$ and delta $F = ((R-R_{neg})/R_{neg})$. The measures are performed on a Packard apparatus (:337 nm laser excitation, simultaneous time resolved measurements on two channels 665 nm ,620 nm).

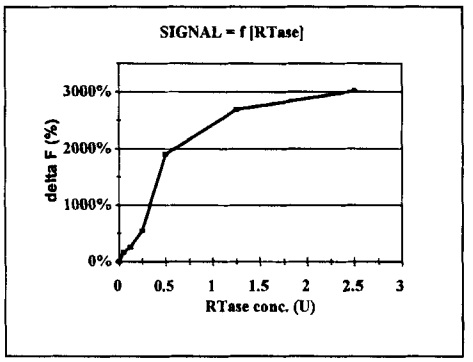
DNase assay format: This assay was developed using a DNA substrate (complementary ODNs respectively 5'-labelled with $TBPEu^{3+}$ and biotin). In the absence of DNase after addition of streptavidin-XL665 conjugate (SA-XL), an emission of XL665 due to energy transfer is observed after excitation of the TBPEu3+ moiety. In the presence of DNase the substrate is degraded into fragments unable to generate energy transfer. The enzymatic

reaction was performed directly in black microtiter plates in 50 µl volume by adding 0.7 ng of substrate to the DNase I dilution and incubating at 37°C for 1 hour.



The reaction was quenched by adding 250 µl of revelation solution containing SA-XL in PO_4 buffer 0.1 M, 0.1% BSA, 0.4M KF for the generation of the specific signal. As shown on the graph the signals obtained for a serial dilutions of DNase I are correlated with the enzyme concentration.

RTase assay format: This assay is based on the synthesis of a cDNA strand from polyA as RNA template, using a 5'-end labelled primer (dT_{20}- $TBPEu^{3+}$) in presence of biotin-16-dUTP. The cDNA formed incorporates biotin moieties statistically along the sequence. SA-XL is then added in the detection mixture to evaluate the RTase activity via energy transfer. The assay was performed using Moloney Murine Leukemia Virus (M-MuLV) as follows: To 19.5 µl of the RTase buffer containing 1.25 µM biotin-16-dUTP, 5.0 µM dTTP, 1.25 mM DTT, 2.5 µg/ml polyA, 3.9 µg/ml oligo dT_{20}-TBPEu3+, 2.5 U M-MuLV RTase was added. After 1 hour incubation at 37°C and 30 min at 56°C, the reaction mixture was diluted 10 times in PBS. 10 µl was transfered to a black microplate followed by 250 µl of SA-XL (5 nM). The measures were performed after 10 min incubation. The graph shows the curve obtained for a serial dilution of Rtase. Quantitation, activity or Rtase inhibitors can therefore be studied using this assay.



CONCLUSIONS: Our homogeneous technology appears versatile and simple. The assays are well suited for automation for example in high throughput screening.

REFERENCES

1. Mathis G., *Clin. Chem.*, **1995**, *41*, 1391-1397

2. Alpha B., Lehn J.-M., Mathis G., *Angew. Chem. Int. Edn. Engl.*, **1987**, *26*, 266-269