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## **Nucleosides, Nucleotides and Nucleic Acids**

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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**

**Alpha-Bazin Béatrice\*, Mathis Gérard.**

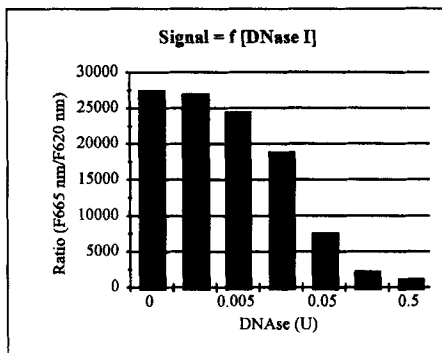
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**ABSTRACT:** An homogeneous non isotopic technique based on time-resolved fluorescence, europium trisbipyridine cryptate (TBPEu<sup>3+</sup>) as fluorescent donor and non radiative energy transfer to an acceptor (cross-linked allophycocyanine, XL665) 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**<sup>1,2</sup>: Our Time-Resolved Fluorescence technique is based on the amplification of the long-lived fluorescence of TBPEu<sup>3+</sup> 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<sup>3+</sup>, 665 nm for XL665) allows to get rid of the media transmission by measuring the ratio of fluorescence : ( $R = F_{665\text{nm}} / F_{620\text{nm}}$  and  $\Delta F = ((R - R_{\text{neg}}) / R_{\text{neg}})$ ). The measures are performed on a Packard apparatus (:337 nm laser excitation, simultaneous time resolved measurements on two channels 665 nm ,620 nm).

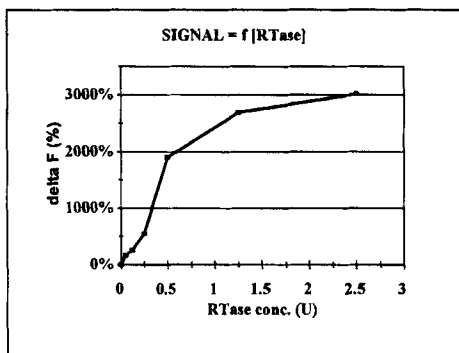
**DNase assay format:** This assay was developed using a DNA substrate (complementary ODNs respectively 5'-labelled with TBPEu<sup>3+</sup> and biotin). In the absence of DNase after addition of streptavidin-XL665 conjugate (SA-XL), an emission of XL665 due to energy transfer is observed after excitation of the TBPEu<sup>3+</sup> 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  $\mu$ l 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  $\mu$ l of revelation solution containing SA-XL in PO<sub>4</sub> 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<sub>20</sub>-TBPEu<sup>3+</sup>) 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  $\mu$ l of the RTase buffer containing 1.25  $\mu$ M biotin-16-dUTP, 5.0  $\mu$ M dTTP, 1.25 mM DTT, 2.5  $\mu$ g/ml polyA, 3.9  $\mu$ g/ml oligo dT<sub>20</sub>-TBPEu<sup>3+</sup>, 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  $\mu$ l was transferred to a black microplate followed by 250  $\mu$ l of SA-XL (5 nM). The measures were performed after 10 min incubation. The graph shows the curve obtained for a serial dilution of Rtas. Quantitation, activity or Rtas 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.

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