TRF Performance of Thermo Scientific Appliskan™ with Europium Sensitivity Assay

This application note describes the TRF performance of Thermo Scientific Appliskan, a filter-based multimode microplate reader. The TRF performance was defined by the europium (Eu) sensitivity assay.

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Introduction

Time-resolved fluorescence (TRF) is a special form of fluorescence with a delay time between the excitation of the sample and the emission detection. The labels used in TRF are fluorescent lanthanide chelates, rare earth metals (Eu, Tb, Sm, Dy) with a specific ligand molecule. Lanthanide chelates have a very long fluorescent lifetime compared to traditional fluorescent labels, which makes them excellent labels for TRF. The fluorescence of the traditional fluorescent labels decays in nanoseconds, whereas the fluorescence of the lanthanide chelates can last for hundreds of microseconds. Lanthanide chelates also have a very large Stoke's shift (the difference between excitation and emission wavelengths) and their emission peak is always very sharp, which makes them good labels for multianalyte assays.

The delay time in TRF measurements reduces the effect of background interference. The background fluorescence originates from plate materials, reagent components, biological samples etc., and most of it decays very rapidly during the TRF delay time. Therefore TRF is a way to separate the fluorescence of the label from the background fluorescence. When the background fluorescence is reduced, the sensitivity of the assay is increased.

The TRF performance of Appliskan was determined by the europium (Eu) sensitivity assay. Europium is the lanthanide ion used chiefly in TRF measurements.

Material and methods

The sensitivity measurements were performed on white Thermo Scientific Microlite 1+ 384 square

well plates with the dilution series of europium (Europium Atomic Absorption Standard Solution, Sigma-Aldrich, USA) ranging from 1 to 10 000 000 amol/100 µl. Europium was diluted in DELFIA Enhancement Solution (Perkin Elmer, Wallac, Finland). The volume of the europium dilution per well was 80 µl. The excitation filter was 340 nm (HBW 80 nm) and the emission filter 616 nm (HBW 8.5 nm). The TRF delay time was 400 µs, integration time 900 µs and the measurement time 1500 ms. The comparison of the assay results was made with ten different Appliskan units from two production lots.

Results

The europium sensitivity of the assay was calculated by the formula:

Theoretical sensitivity = (3 x SD_{blank} / $I_{ref-blank}$) x c_{ref},

where "ref" was the dilution 1.0 pmol/100 µl and I was the signal in relative fluorescence units (RFU). The dynamic range was calculated by the formula:

Dynamic range = log ($Max_{mean-blank}$ / 3 x SD_{blank}),

where "Max" was the highest signal of the dilution series.

The theoretical europium sensitivity of the assay was calculated for all ten Appliskan units and the average of those ten units was 4.5 amol/well. The dynamic range was > 5.0 decades (Table 1). The linearity of the assay is shown in Figure 1.

The technical specifications of Appliskan define the TRF sensitivity of the instrument to be < 20 amol europium/well with a 384-well plate. As the comparison of the results of ten Appliskan units shows, the average sensitivity was < 5 amol/well. Therefore the typical sensitivity value, which is reached with Appliskan, is in practice always clearly under the guaranteed specification value of the instrument.

Table 1. Sensitivity and dynamic range of ten Appliskan units (average value).

Theoretical sensitivity (amol europium/well)	Dynamic range (decades)
4.5	> 5.0

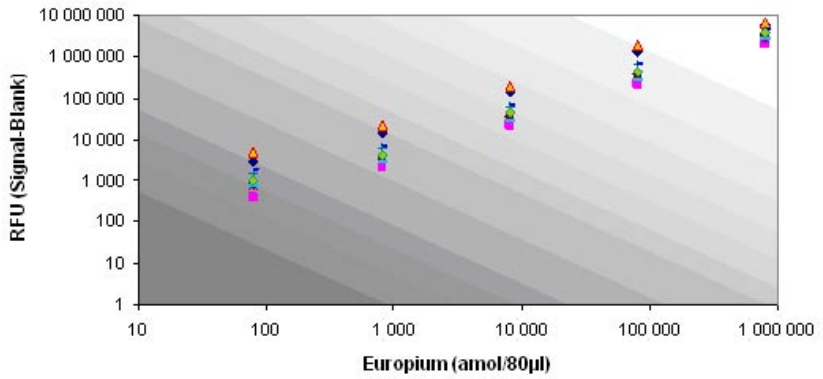

Figure 1. Linearity of the assay with ten Appliskan units

Conclusions

Even though Appliskan has a very good performance with all of its five detection technologies, this europium assay shows that excellent sensitivity values can especially be reached with the TRF detection mode.

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