A Homogeneous TR-FRET NF-κB Protein:DNA Binding Assay

Introduction

Nuclear Factor-κB (NF-κB) is a transcription factor that is considered to be of physiological importance because of its key role as a regulatory molecule involved in immune response, inflammation, cancer and apoptosis.1,2

We have developed a time resolved-fluorescence resonance energy transfer (TR-FRET) assay to evaluate the binding interaction between the p65 subunit of NF-κB and a dsDNA NF-κB-specific (HIV-L) consensus sequence. The development of a TR-FRET NF-κB binding assay has been reported previously using direct labeled p65 specific dsDNA. We have now further developed the assay to incorporate generic europium [Eu(TMT)] donor and Cy5 acceptor reagents (figure 1).

![Figure 1. TF-FRET NF-κB binding assay schematic](image)

Method

Oligonucleotide sequences were prepared ‘in house’ using standard phosphoramidite chemistry and purified by C18 reverse phase HPLC. Double-stranded DNA was prepared by incubating equimolar amounts of the NF-κB specific dsDNA bound to Cy5 labelled streptavidin and purified by C18 reverse phase HPLC. Double-stranded DNA was purified by incubating equimolar amounts of the NF-κB specific dsDNA bound to Cy5 labelled streptavidin and unmodified non-coding strands in a 78°C water bath for 3-5 minutes before allowing to cool to ambient temperature.

Specific biotinylated dsDNA was titrated from 40nM with a constant 10nM p65-GST recombinant protein. Cy5 labelled streptavidin was added to the biotinylated dsDNA at a 1:2 (w/w) concentration. Data plotted as quadruplicates, mean ± SEM.

![Figure 2. NF-κB assay sensitivity of binding.](image)

Evaluation of the assay to DMSO tolerance showed that the cell cytoplasm which inhibits DNA binding activity.

![Figure 3. NF-κB binding competition.](image)

Conclusions

- We have demonstrated the ability to measure NF-κB specific dsDNA binding to a p65-GST recombinant protein by TR-FRET using generic reagents and measuring on FARCyte.
- The assay is both robust and sensitive using generic TR-FRET reagents.
- The results highlight the potential of the FARCyte Fluorescence Plate Reader for measurement of sensitive TR-FRET assays.

References