Video Article

Measuring DNA binding of human p53 using scintillation proximity assay (SPA) beads

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Abstract

Measurement of DNA binding activity can be an important parameter for analysis of the function of transcription factors in normal and diseased tissues. The tumor suppressor p53 is often mutated in cancer and those mutations almost exclusively reside in the DNA binding domain. This protein alters the expression of more than 50 different genes through binding to well characterized regulatory sequences. Methods for measurement of DNA binding are not generally amenable to high throughput screening due to the nature of the separation technology or the expense of the instrument involved. Using scintillation proximity assay (SPA) beads for this can allow researchers to get around this obstacle. SPA beads have been used in a variety of formats to measure different protein functions including levels of specific growth factors and receptors (Khawaja 2007, Carrick et al. 2008, Mannoury La Cour 2009, reviewed in Glickman et al. 2008). The DNA binding protein is provided from a human cell extract or from a heterologously expressed protein from insect cells. The buffer, antibody, 3H-labeled DNA and non-specific DNA are combined in wells of a 96-well plate and then the SPA beads are added. The plate is sealed and inserted into a scintillation or luminescence counter where counting commences after 5 minutes. The plate counts are recorded over the next several hours and the specific counts are calculated by comparing values from wells with protein to those without. Previous work has indicated the assay is sensitive (measures DNA binding of approximately 50 pg of p53), fast (user time involved less than 15 minutes, time to result approximately 2 hours), and specific (specific counts are dependent on the sequence used for labeling). As this approach is a real-time measurement of DNA binding, both on- and off-rates of DNA binding can be determined. Modifications of this approach will be discussed to measure other aspects of protein function.

Disclosures

No conflicts of interest declared.

