

innovations

from the University of Vermont

TITLE: MOLECULAR ACCESSIBILITY ASSAY

INVENTORS: Jeffrey Blaisdell and Susan Wallace

DESCRIPTION: DNA binding proteins play critical roles in all living organisms, notably in pathways known to be affected in certain diseases, such as cancer. An initial step in the characterization of these proteins is purification by affinity chromatography. This typically involves over-expression of the protein and often leads to a fraction of molecules being in a mis-folded (non-active) state. Considering that many studies require an exact quantification of “active” protein molecules, some measurement of activity is needed. This technology provides a novel assay for rapidly determining the binding state of stable protein-DNA interactions based on the molecular accessibility of a fluorescent reporter molecule. Using this assay, the fraction of active protein molecules can be determined rapidly, accurately, and utilizing a high-throughput microplate-based system.

ADVANTAGES: Total time to determine the active fraction of a protein preparation is reduced from the standard 1-2 days of a traditional gel-based method, to less than 1 hour for the accessibility-based method. In addition, neither radioactive nor fluorescent labels are required for target DNA sequences. The molecular accessibility assay can be generalized for use with many types of DNA binding proteins using target specific reporter molecules or fluorophore-tethered nucleic acids and can be modified for various protein/DNA complex experiments including reaction rate analysis, characterization of enzyme active site residues, or binding-site identification to name a few.

PATENT STATUS: Patent pending

LICENSING STATUS: Worldwide rights available

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