C716 APPLICATIONS OF TIME-RESOLVED FLUOROMETRY IN MOLECULAR BIOLOGY TECHNIQUES, Theodore K. Christopoulos and Eleftherios P. Diamandis (Dept. Clin. Biochem. Toronto Western Hosp. Toronto, CANADA M5T 2S8)

A streptavidin-based macromolecular complex labeled with the europium chelate of 4,7-bis (chlorosulfophenyl) 1,10-phenanthroline-2,9 dicarboxylic acid has recently been reported (Anal Chem 1990; 62:1841-1845) for highly sensitive time-resolved fluorescence immunoassays. We now demonstrate that this reagent is very useful for non-isotopic molecular biology techniques. Biotinylated goat anti-mouse antibody (GAMIg) stained with the streptavidin reagent was detected on 1μL spots, on nitrocellulose, down to about 25 pg. Mouse IgG, alpha-fetoprotein, ferritin and carcino-embryonic antigen were detected down to 25 pg on nitro-cellulose by reaction with their specific monoclonal antibodies (except mouse IgG) followed by biotinylated GAMIg and streptavidin. Biotinylated mol. weight protein markers (Sigma) on Western blots gave patterns identical to those obtained with alkaline phosphatase-streptavidin (ALP-SA) and NBT-BCIP substrate. Spotted biotinylated DNA (Vector), was detected down to ~ 2 pg. A λ phage vector, containing the insert λ AmT1 (Amersham) was digested EcoR1 and electrophoresed along with the undigested λ AmT1. After Southern blotting, the digested and undigested fragments were hybridized to a biotinylated (Nick translation) probe (AmT1, Amersham). Biotinylated hybrids were detected in positions identical to those by the ALP-SA method. Biotinylated DNA markers (Hind III digested λ DNA) on Southern blots gave a pattern identical to that by ALP-SA. Plasmid pBR 328 fragments (Boehringer) on Southern blot were also successfully detected by using a linearized biotinylated (Nick translation) pBR 328 plasmid as probe. Similar results were obtained with plasmid pUC18. Experiments with the polymerase chain reaction revealed that amplified products, in the presence of biotinylated dUTP, could also be detected on Southern blots with the new reagent. In all experiments the final fluoresecent complex was examined qualitatively on a UV transilluminator and also quantified with a time-resolved fluorometer working as a high resolution laser scanner (CyberFluor Inc.). We conclude that this new system (instrument + chemistry) may find many applications in the field of biotechnology.