M15

A STREPTAVIDIN BASED MACROMOLECULAR COMPLEX LABELED WITH A EUROPIUM CHELATOR AS A UNIVERSAL DETECTION SYSTEM IN TIME-RESOLVED FLUORESCENCE IMMUNOASSAY. R. C. Morton, and E. P. Diamandis (CyberFluor Inc., 179 John Street, Toronto, Ontario, Canada, M5T 1X4)

We have recently described the synthesis of a new europium chelator, EuroFluor S® [4,7-bis(chlorosulfophenyl)-1, 10-phenanthroline-2,9 dicarboxylic acid] and methods for labeling antibodies, avidin and streptavidin. A maximum of 15 EuroFluor S® molecules can be directly introduced into streptavidin (SA) without loss of its binding activity. We have also devised a new method for multiple fluorescence labeling of SA in which SA is linked to bovine thyroglobulin (TG) which carries approximately 150 molecules of EuroFluor S®. No quenching effects were observed due to multiple labeling. We describe here a method for generating a macromolecular complex from the covalently linked streptavidin-thyroglobulin conjugate [SA-TG-(EuroFluor S®)$_{150}$], when it is treated with europium ions, in the presence of excess EuroFluor S® labeled TG. This new reagent enhances the sensitivity of detection of a model prolactin immunoassay (based on a 'sandwich' principle, with biotinylated antibodies as complementary reagents) by a factor of 5-fold compared to the assay which uses SA-TG-(EuroFluor S®)$_{150}$. This new reagent was found to be stable for at least 1 year at 4° C when stored at a concentration of 15 mg/L. The reagent is used after 50-fold dilution in a buffer diluent. The diluted reagent was shown to be stable for at least 1 month at 4° C. We conclude that this new reagent is a universal tracer system for time-resolved fluorescence immunoassays having sensitivity in the 10^{-11}-10^{-13} mol/L concentration range.