

Effect of EDC on Oligonucleotide Coupling Efficiency

Twenty-three amine-C12-modified oligonucleotide probes were coupled to individual carboxylated microsphere sets using freshly-opened EDC. Coupling was performed according to our standard Oligonucleotide Coupling protocol. Coupling was repeated after the EDC had been stored desiccated at -80°C for 3 months. Coupled microspheres were resuspended in TE, pH 8.0 and stored at 4°C in the dark. Coupling efficiency was functionally assessed by direct hybridization to 23 biotinylated target sequences amplified from genomic DNA. Hybridization was performed according to our standard DNA Direct Hybridization protocol. The net median fluorescent intensity for each probe-coupled microsphere set was determined and used to calculate the percentage of the signal obtained on the “fresh EDC”-coupled microspheres. The achievable signal was decreased by 13.5 to 84% (37% average) for 22 of 23 of the microsphere sets coupled using the stored EDC.

