Alkaline phosphatase activity assay based on transcription of light-up RNA aptamer

이서영, 김한솔, 박연경, 박기영, 박현규†
한국과학기술원
(hgpark@kaist.ac.kr†)

We, herein, describe a novel alkaline phosphatase (ALP) activity assay based on the transcription of light-up RNA aptamer. In this strategy, phosphate probe (PP) is dephosphorylated by ALP, hybridized with template probe (TP), and then extended by DNA polymerase to make double-stranded DNA (dsDNA) product, which contains double-stranded T7 promoter sequences and malachite green aptamer (MGA) sequences. By the activity of T7 RNA polymerase, a large number of MGAs, that specifically bind to malachite green (MG), are amplified from dsDNA product, consequently highly enhancing the fluorescence of MG. Based on this principle, we successfully determined ALP activity down to 0.000018 U/mL with excellent selectivity. The practical applicability of this strategy is also demonstrated by reliably determining ALP activities in the human serum.