Electronic Supplementary Material (ESI) for Nanoscale.

Supplementary Material

Real-Time Investigation of Human Topoisomerase I Reaction Kinetics using an Optical Sensor: A Fast Method for Drug Screening and Determination of Active Enzyme Concentrations.

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Figure S1. Determination of the concentration of total hTopI.

A comassie stained SDS-polyacrylamide gel containing a titration of bovine serum albumin (BSA) ranging from 1000-62.5 ng (lanes 1-5), a size marker (lane 6) and 5µL of the purified hTopI fraction used for the experiments (lane 7). The relative intensity of the bands corresponding to the BSA or hTopI is shown in the gel below. These values were used to calculate the concentration of total hTopI (22 ng/µL).

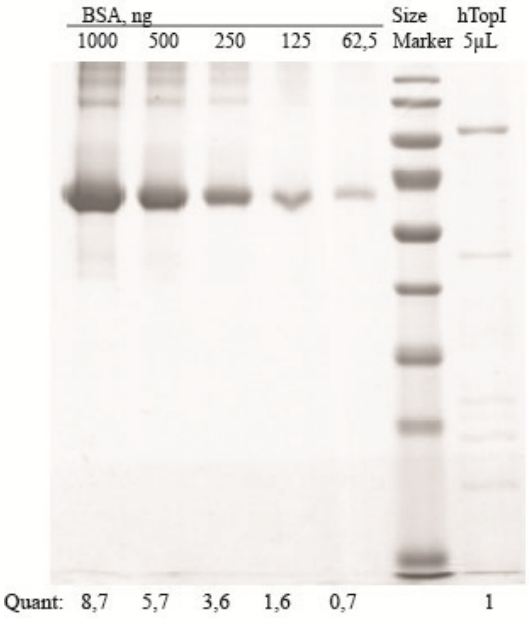

Figure S2. Conversion of fluorescence to product concentration.

To generate a conversion curve that allows the fluorescence readout to be translated into a product concentration a known concentration of a synthetic product (a DNA sensor without the tri-nucleotide coupled quencher, (which is cleaved off by hTopI on the sensor substrate)), was incubated under standard reaction conditions. The fluorescence of the reaction product was measured in the qPCR machine, and plotted as the function of the concentration. The conversion constant at the specific reaction conditions was estimated to 3.7×10^{-6} µM/fluorescence unit measured by making a linear least square fit to the data points.