

**C** Dose dependence of a known inhibitor by two different ATPase methods



**Supplemental Figure 1.** Characterization of the white plate, fluorescent assay. (A) Determination of the kinetic parameters of DnaK-DnaJ in the fluorescence method, under the screening conditions. (B) Determination of the linear range. The concentration of DnaK was 0.4  $\mu$ M and DnaJ was 0.7  $\mu$ M. (C) Direct comparison of the IC<sub>50</sub> values of a known inhibitor in the normal, malachite green-based absorbance assay and the white, fluorescent plate assay. The concentration of DnaK was 0.4  $\mu$ M, DnaJ was 0.7  $\mu$ M and ATP was 1 mM. Results are the average of triplicates and the error bars represent standard error of the mean.

corning 3825 low volume



**Supplemental Figure 2.** Occasional outliers in white, opaque mirotiter plates. Stacks of untreated microtiter plates were monitored for fluorescence (ex. 430 nm. em. 530 nm). Note that occasional plates show significantly different fluorescence patterns and that all the plates show a "sharktooth" pattern. Representative data sets are shown for three manufacturers. Based on these results, we chose Greiner 784075 low volume plates for our experiments.