Supplementary Material

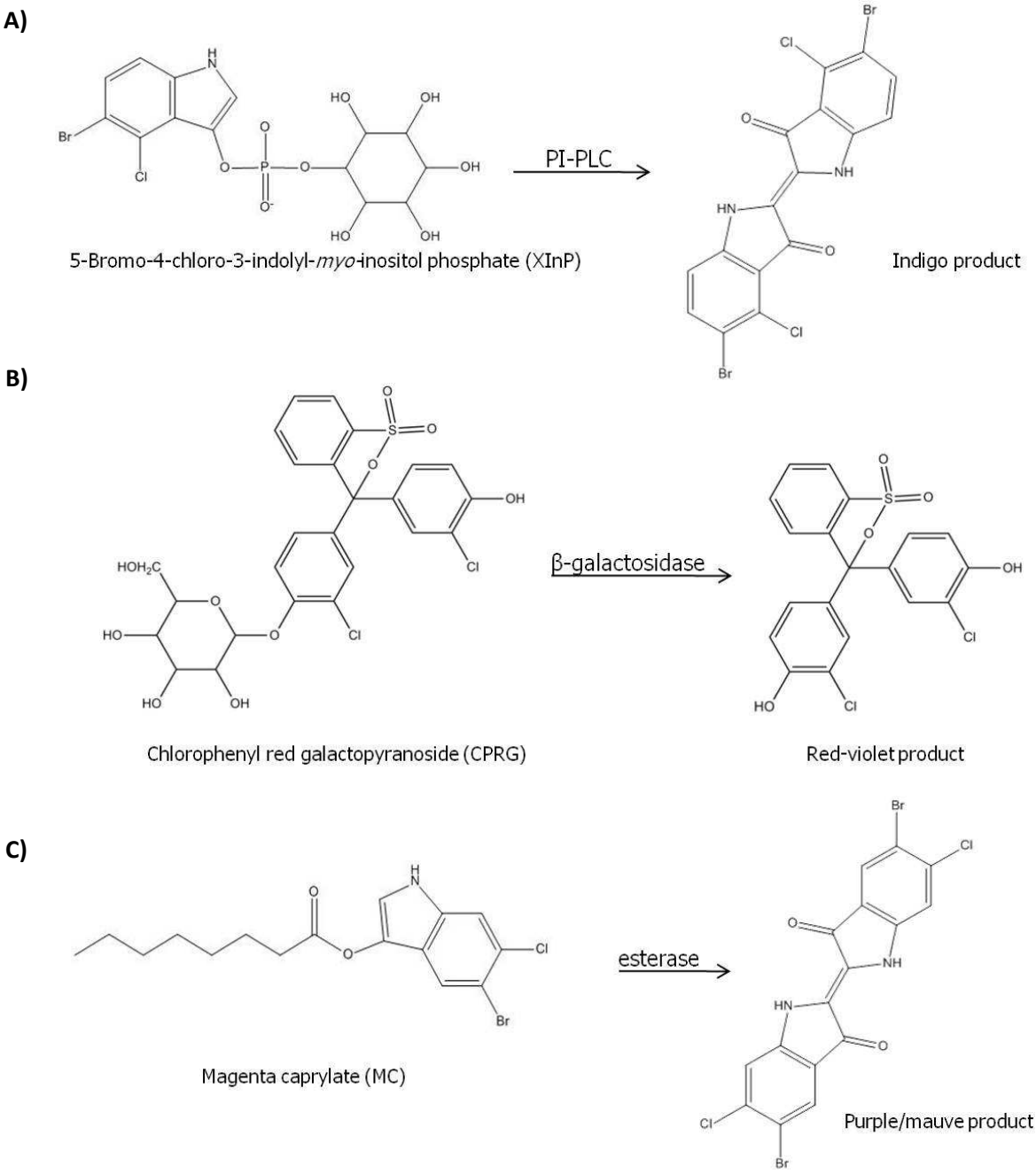

Figure 1S. Schematics showing the enzymatic reactions of PI-PLC, galactosidase, and esterase with **(A)** 5-bromo-4-chloro-3-indolyl-*myo*-inositol phosphate **(B)** chlorophenyl red galactopyranoside **(C)** magenta caprylate, respectively.

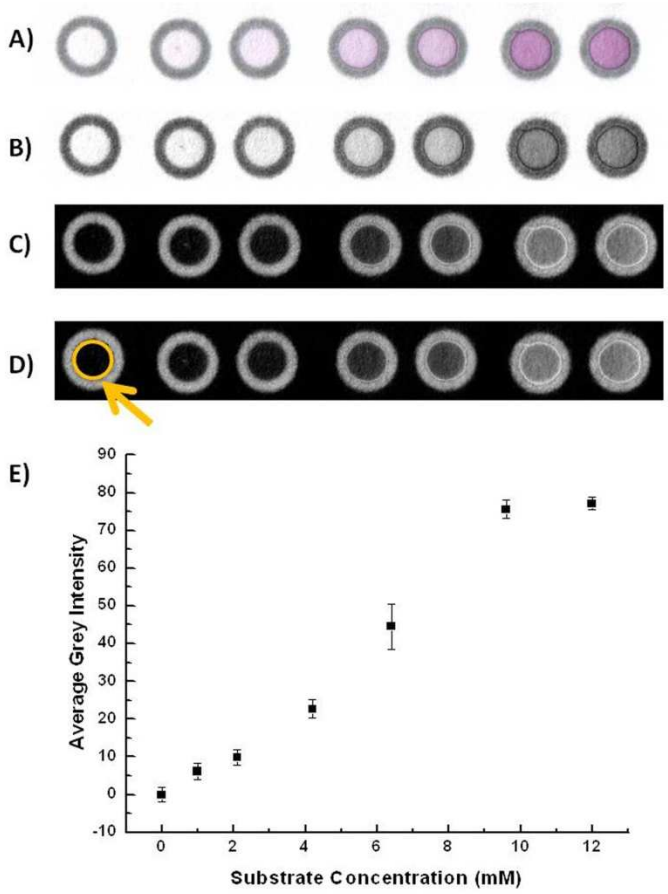

Figure 2S. Protocol for ImageJ analysis. A) A digitial image of the paper device is generated using a flat-bed scanner. B) Using ImageJ, the image is converted to 32-bit grey scale. C) The image is then inverted. D) The spot area is selected individually, and the grey intensity is measured.

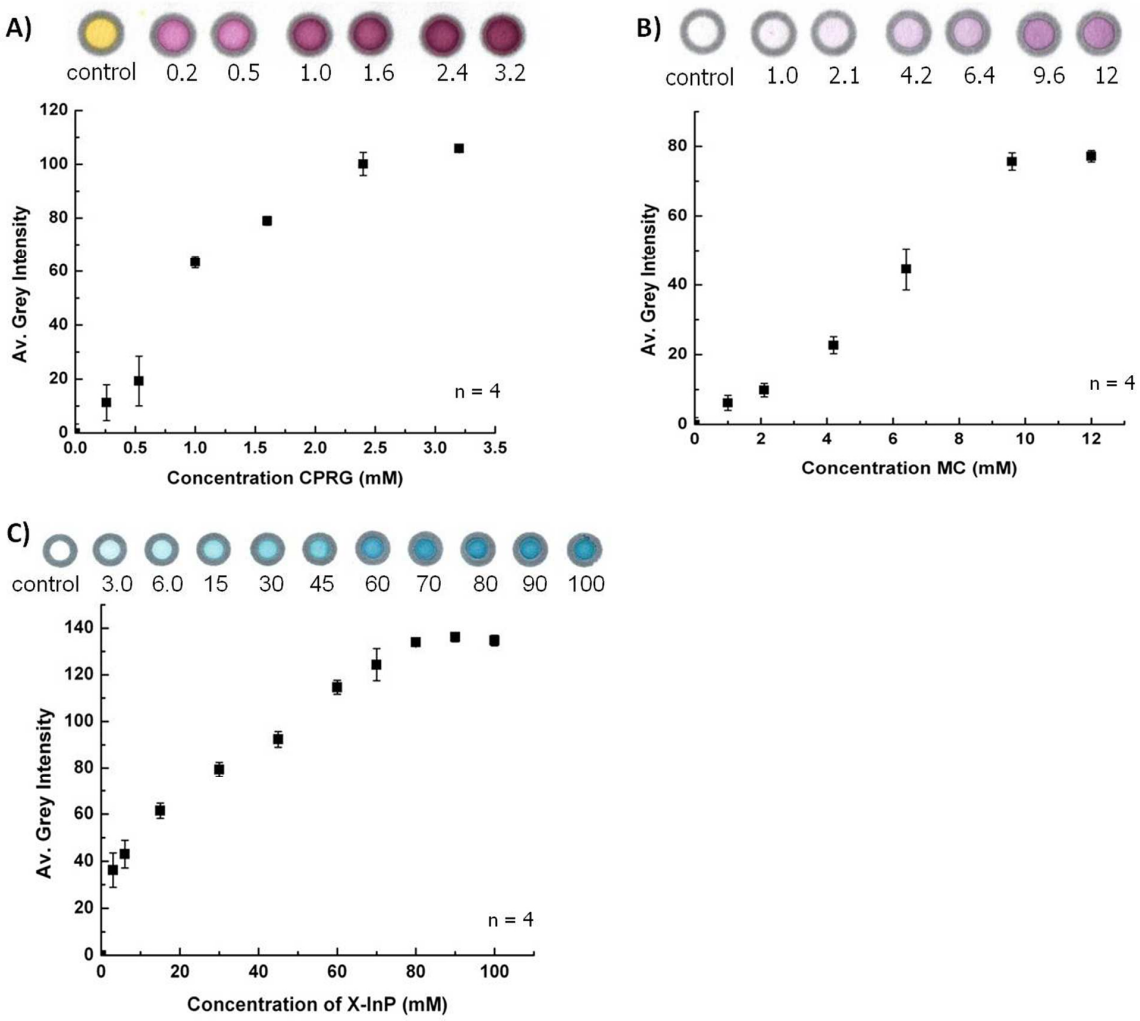

Figure 3S. Determination of optimal substrate concentrations for A) CPRG B) MC and C) X-InP using the corresponding enzymes. In each paper device, a constant amount of the appropriate enzyme was used while the concentration of substrate (in mM) was increased. A negative control for each assay, in which no enzyme was present, is shown as the first well. The average grey intensity was measured and plotted versus substrate concentration, where each data point represents the average (± standard deviation) grey intensity of four measurements. The optimal concentration was determined from the maximum grey intensity generated for each assay.

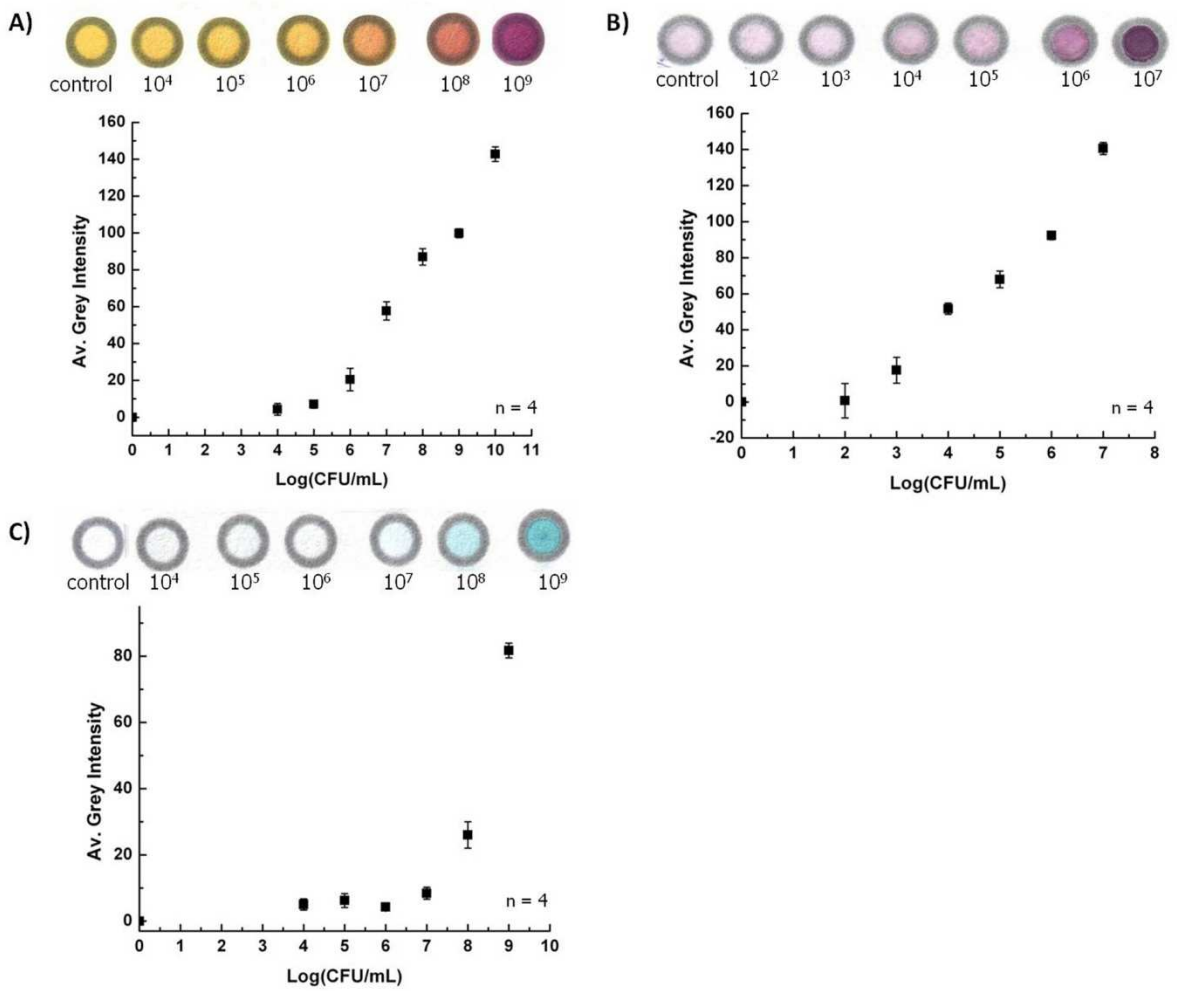

Figure 4S. Determination of the limit of detection for each live bacterial assay. Pure cultures were enriched overnight with shaking. Serial dilutions were made in buffer from the bacterial samples, and each dilution was tested on the paper device for enzyme activity and average grey intensities were measured. The limit of detection for A) *E. coli* O157:H7 B) *S.* Typhimurium and C) *L. monocytogenes* was estimated to be 10^6, 10^4, and 10^8 CFU/mL, respectively. However, enzyme activity and concentration of cells do not directly correlate since target enzyme may accumulate over the long enrichment period.

The limit of detection was determined for each assay. Isolated colonies were enriched for overnight, to ensure a high concentration of cells, and serial dilutions were made in lambda buffer. A sample of each dilution was tested on the paper devices and plated for validation of bacterial cell concentration. The results of this study, including the grey intensity analysis, are shown in Figure 3S. The limit of detection (LOD) for esterase occurs at 10^4 CFU/mL concentration of *S.* Typhimurium, while

the LODs for β-galactosidase and PIPLC occur at 10^6 and 10^8 CFU/mL, respectively. These studies provided a baseline for determining the concentration of bacteria necessary in the enrichment media to allow detection of bacteria from food samples; however, because the bacteria were enriched overnight, an accumulation of the target enzymes can be expected, and the measured enzyme activity from these samples does not directly correlate with the concentration of cells present. Differences in LODs are most likely due to differences in the expressed enzyme levels as well as the molar absorbtivities of the dyes used in these experiments.