

Supplementary Information

3D-Printed Fluidic Devices Enable Quantitative Evaluation of Blood Components in Modified Storage Solutions for Use in Transfusion Medicine

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Figure S1. Design of the fluidic device

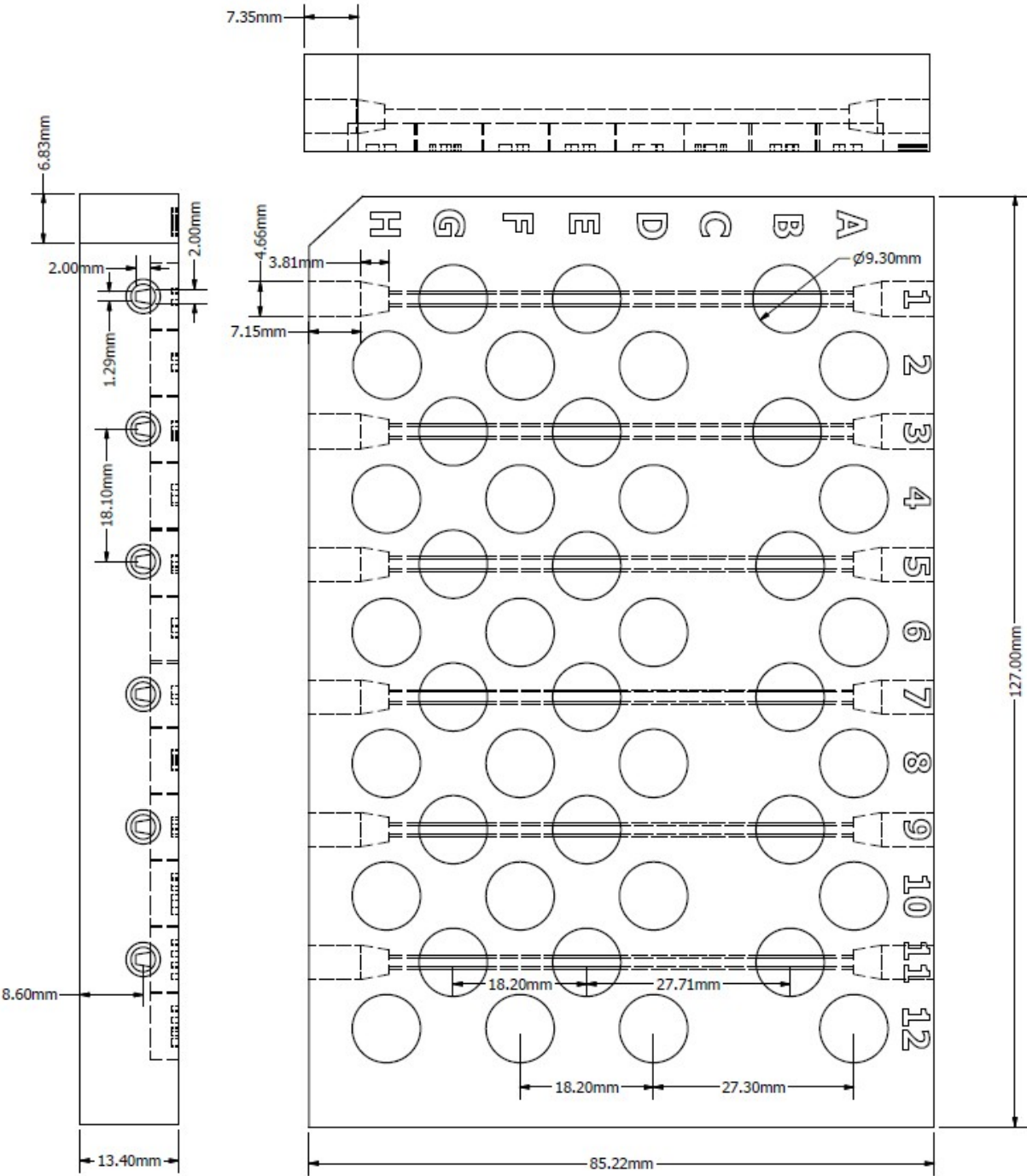


Figure S1. A schematic of the device from 3 different viewpoints.

Figure S2. Validation of the alignment of the device and printed wells in the plate reader

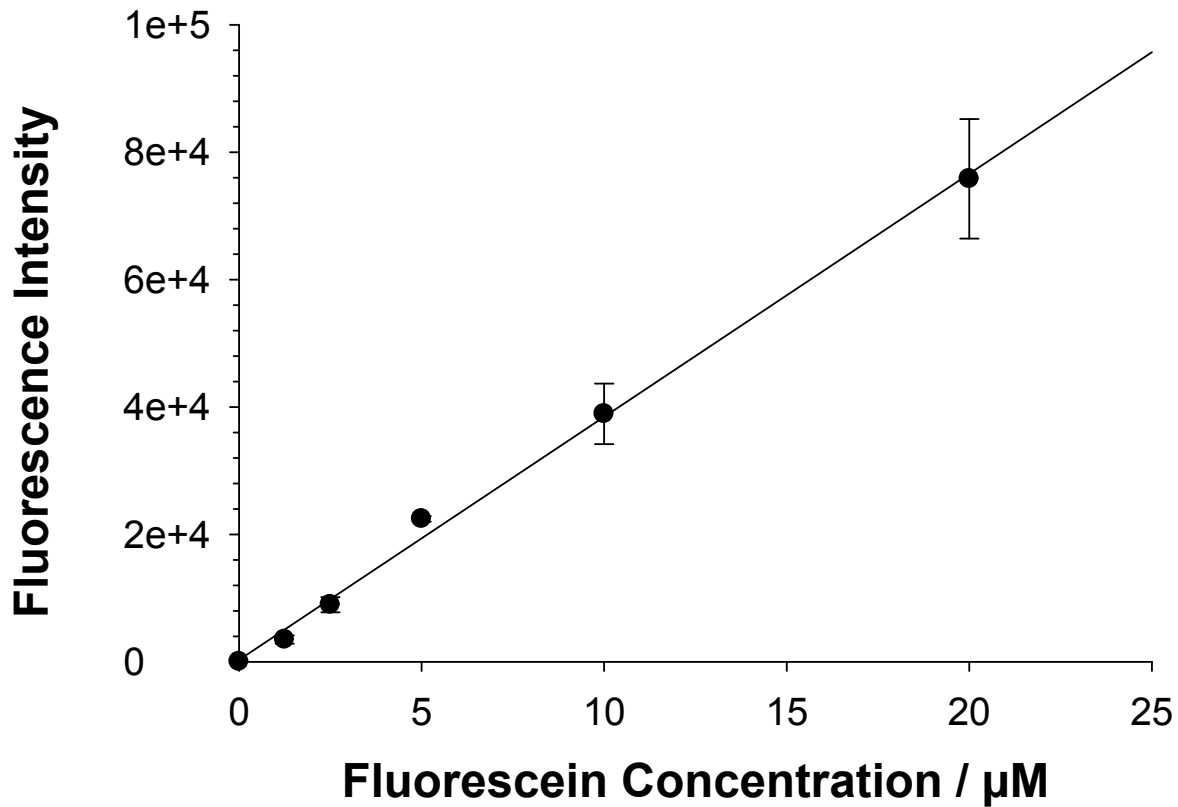


Figure S2. Six fluorescein solutions (concentrations ranging from 0 to 20 μM) were prepared and loaded into the six inserts in the wells in row B above each channel. Fluorescence intensity above these inserts was detected using a plate reader and plotted versus fluorescein concentration. The linearity ($R^2 = 0.99$) shows that the device and wells are properly aligned with the plate reader. $n = 3$, Error = std dev.

Figure S3. Graphical representation of data in Table 2 evaluating optimal collection time for ATP

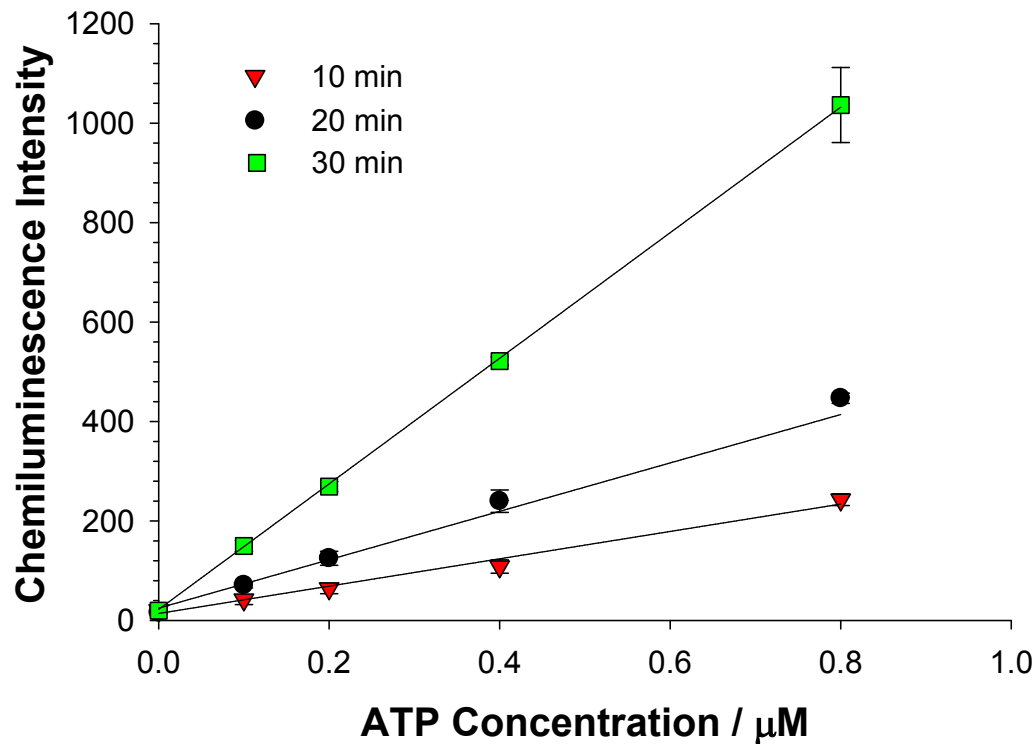


Figure S3. Chemiluminescent intensity as a function of ATP concentration and the amount of time (10, 20, or 30 min) that ATP was allowed to diffuse from the channel, through the membrane pores of the insert and into the well before measurement. While the 30 min collection time provided optimal sensitivity, the 20 min collection time provided the required sensitivity for the actual ATP that was released from the ERYs.

Figure S4. Graphical representation of the data in Table 3 evaluating optimal volumes of chemiluminescent reagents

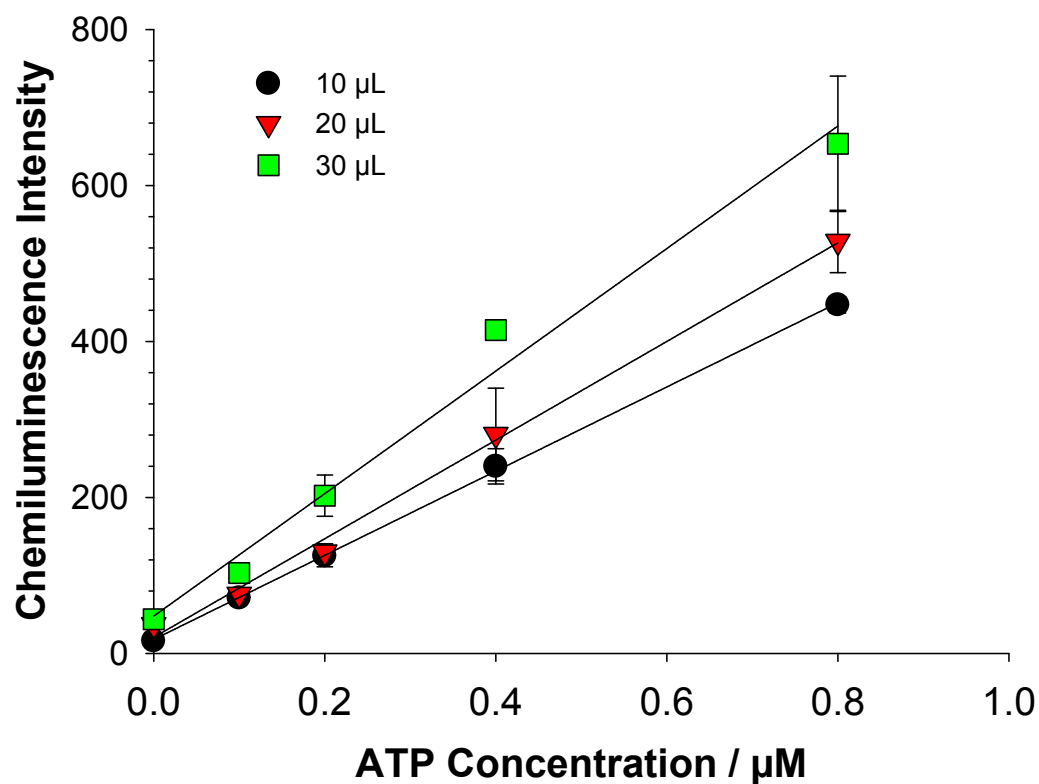


Figure S4. Chemiluminescent intensity as a function of concentration and volume of luciferin/luciferase mixture used to generate emission. We chose 10 μL because it provided an improved coefficient of correlation and reduced background emission.

Figure S5. An ATP calibration curve with optimal conditions

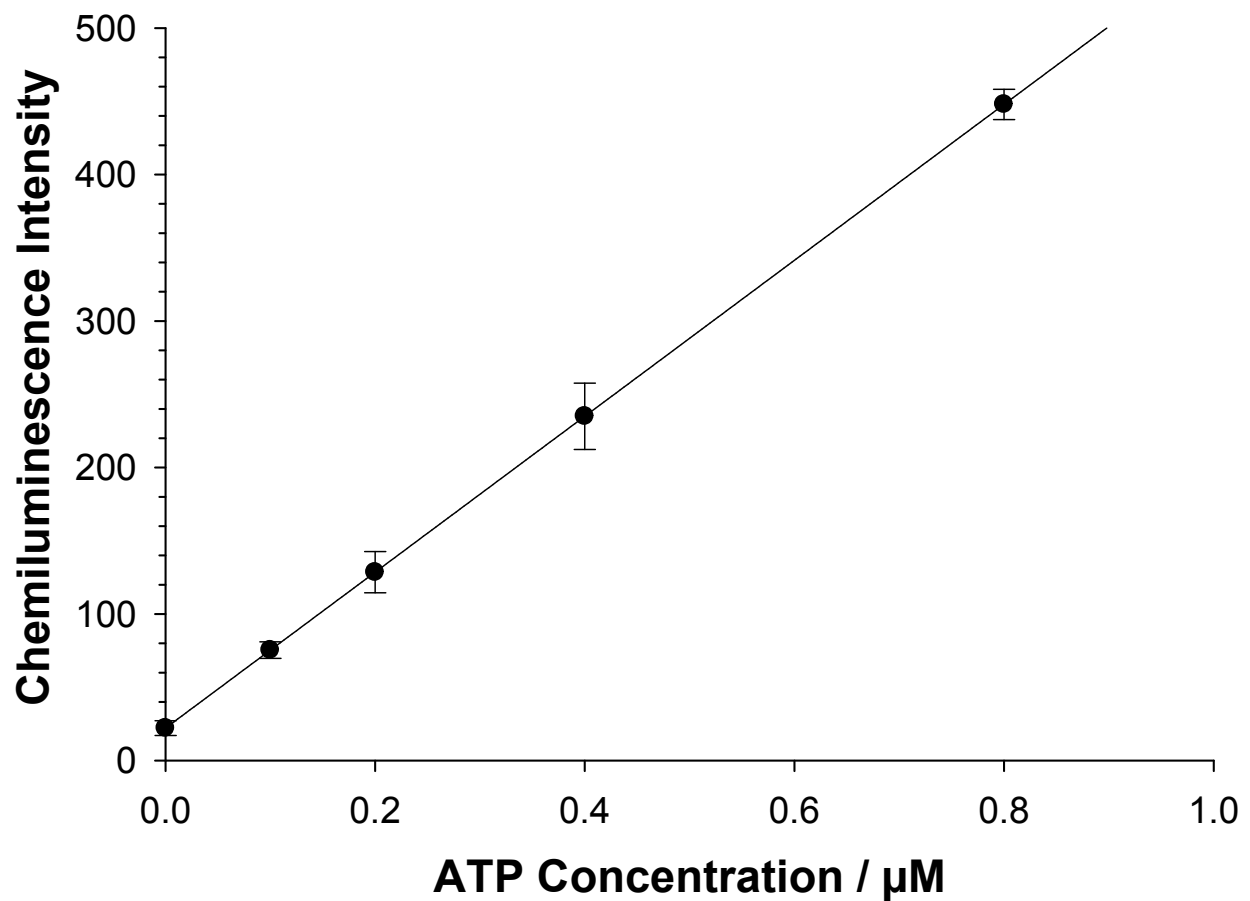


Figure S5. A calibration plot for different concentrations of ATP. Collection time for ATP prior to measurement was 20 min, while 10 μL of luciferase/luciferin assay was used for the chemiluminescence assay. The Y-axis shows calibrated chemiluminescence intensities from inserts in wells in row B above each channel. A standard of 20 nM ATP was added to well 2A to account for possible errors from instrument or changes in luciferin/luciferase concentration. ($n = 3$, $R^2 = 0.99$, Error = std dev)

Figure S6. Chemiluminescence intensity in inserts vs. ATP concentration flowing in corresponding channel

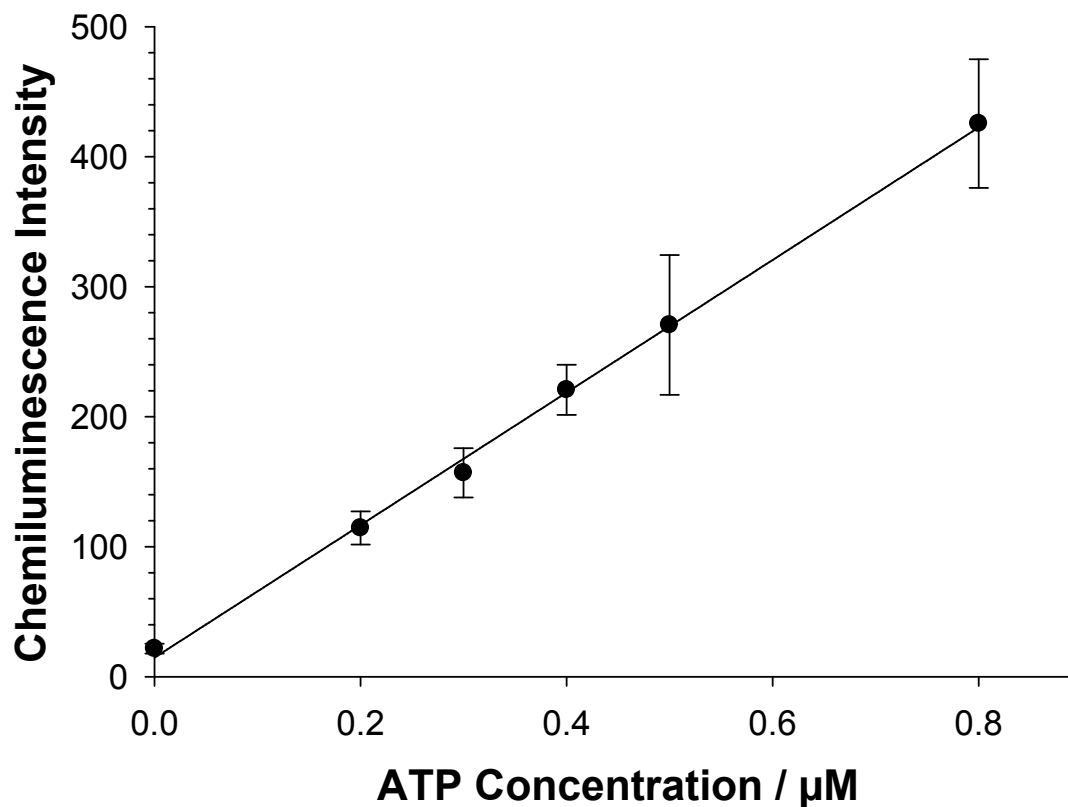


Figure S6. An analytical calibration plot for different concentrations of ATP. Six ATP solutions were simultaneously circulated in a random order in six channels. The Y-axis depicts the chemiluminescence intensity of collected ATP in the inserts from the wells in row B above each channel, while the X-axis shows the concentrations of ATP that was pumped through the corresponding channel. ($n = 5$; $R^2 = 0.99$; Error = std dev)

Figure S7. One-step quantification of ATP released from ERYs stored in AS-1N and AS-1

This study investigated the high-throughput potential of the 3-D printed device in a real application. Briefly, ATP standards and two samples (ERYs stored in AS-1N and AS-1) were simultaneously circulated on the same device so that calibration and sample quantification can be achieved in a single step; this not only improved measurement efficiency, but also increased precision (i.e., by reducing errors resulting from equipment shift or assay degradation between separate calibration and measurement).

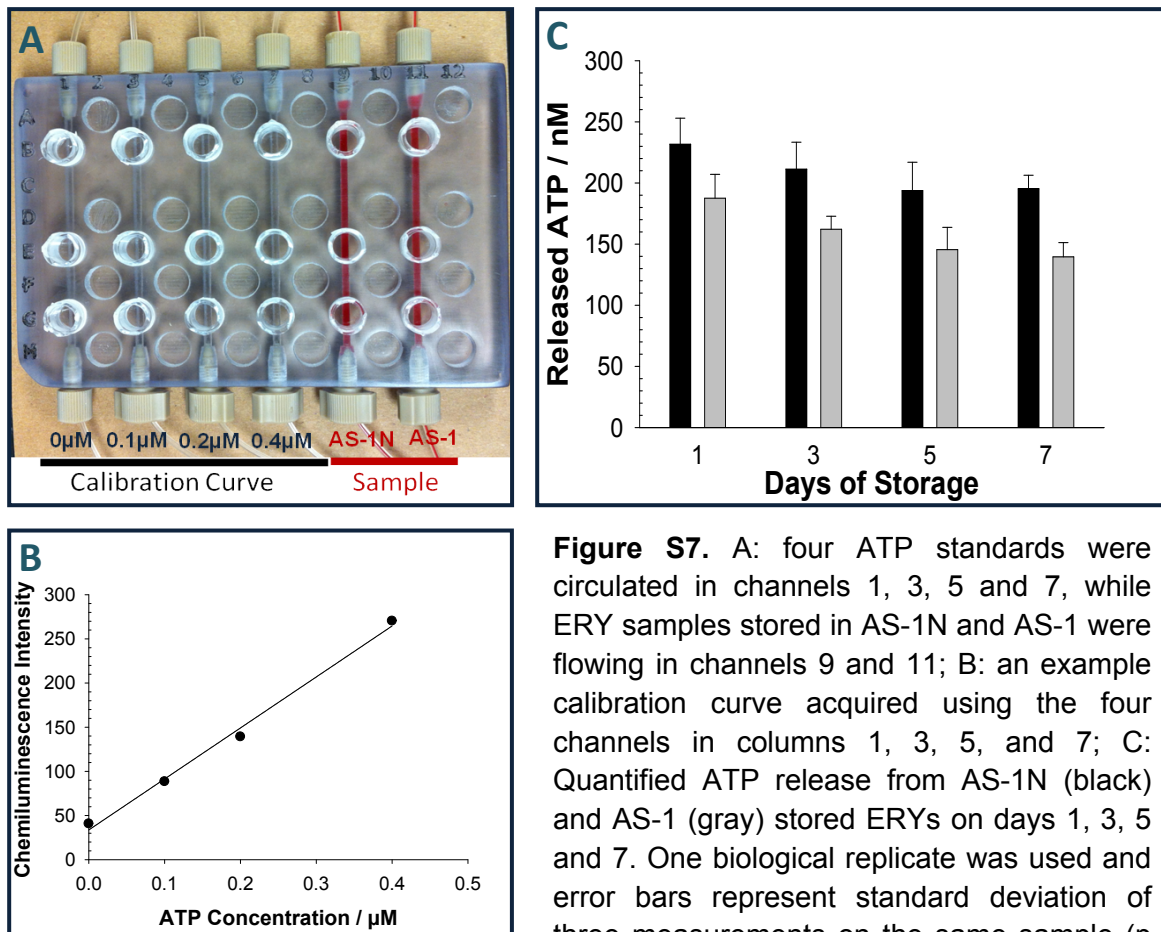


Figure S7. A: four ATP standards were circulated in channels 1, 3, 5 and 7, while ERY samples stored in AS-1N and AS-1 were flowing in channels 9 and 11; B: an example calibration curve acquired using the four channels in columns 1, 3, 5, and 7; C: Quantified ATP release from AS-1N (black) and AS-1 (gray) stored ERYs on days 1, 3, 5 and 7. One biological replicate was used and error bars represent standard deviation of three measurements on the same sample ($p < 0.05$ for all 4 groups).