1. Solution Plate 1 Layout



1. Solution Plate 2 Layout



C. Test Plate Layout



Supplemental Figure 1. Template for the layout for feeder plates and test plate for single concentration testing phase. Each feeder plate (A and B) is filled with aliquots of test chemicals (1 replicate of each of 72 test agents per feeder plate, black text), a 12 concentration series of standard TPO inhibitor methimazole (MMI; one well per concentration, green text), a single high concentration (final assay concentration 87.5µM) of each of 6 reference chemicals (one well each of resorcinol – RSC; 2,2′,4,4′-tetrahydroxybenzophenone - BP2; dibutyl phthalate – DBP; sodium perchlorate - NaPer; sulfamethazine – SMZ; and triclosan – TSC), 4 wells of DMSO control (for inhibitor free enzyme control), and 2 wells of deionized water (for enzyme-free blank). To load test materials into each 384 well assay plate, 2.1 μl from each of 96 wells of a feeder plate are transferred simultaneously into one of 4 X 96 well templates in the assay plate. 2 feeder plates are used to populate the assay plate such that each well of each feeder plate results in 2 replicate wells in each assay plate (eg. yellow and blue wells in assay plate in C are populated from feeder plates 1 (A) and 2 (B), respectively). As similar strategy was used for the multiple concentration phase AUR-hPTO assay.