**Alginate Immobilization of Metabolic Enzymes (AIME) for High-Throughput Screening Assays**

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The EPA's ToxCast program utilizes a wide variety of high-throughput screening (HTS) assays to assess chemical perturbations of molecular and cellular endpoints. A key criticism of using HTS assays for toxicity assessment is the lack of xenobiotic metabolism (XM) which precludes both metabolic detoxification as well as bioactivation of chemicals tested *in vitro* thereby mischaracterizing the potential risk posed by these chemicals. To address this deficiency, we have developed an extracellular platform to retrofit existing HTS assays with XM activity. This platform utilizes the S9 fraction of liver homogenate encapsulated in an alginate gel network which reduces the cytotoxicity caused by direct addition of S9 to cells in culture. Alginate microspheres containing encapsulated human liver S9 were cross-linked to solid supports extending from a 96-well plate lid and were assayed using a pro-luciferin substrate specific for CYP3A4 (IPA). We demonstrate that S9 was successfully encapsulated and remained enzymatically active post-encapsulation with 5-10X the CYP3A4 activity as compared to 1 µg solubilized human liver S9. Ketoconazole, a known inhibitor of human CYP3A4, inhibited CYP3A4 activity in a concentration-dependent manner ($IC_{50}$: 0.27 µM) and inhibition was similar to that of solubilized S9 ($IC_{50}$: 0.15 µM). Inhibition of CYP3A4 with ketoconazole and the passive diffusion of IPA and the resulting D-luciferin metabolite demonstrates small molecule permeability into and out of the microsphere which is a necessary requirement for testing ToxCast chemicals with this platform. Overall, these results demonstrate that HTS assays may be retrofitted with XM activity using immobilized alginate-S9 microspheres. *This abstract does not necessarily reflect the policy of the US EPA.*