Liquid-Liquid Extraction for isolation of 6-Chloronicitinoic Acid and 6-Hydroxynicitinoic Acid:

Materials Needed:
1.5mL Eppendorf Tubes
2 mL Eppendorf Tubes
10mL glass test tubes
Ethyl Acetate
12M Hydrochloric Acid
pH paper
Centrifuge
Vortex
Pipet

Protocol:

1) A 1mL fraction of a liquid-cell culture of e.coli, expressing an enzyme of choice in M9 media (with or without the substrate the enzyme degrades) is taken and centrifuged and the supernatant transferred to a 1.5mL Eppendorf tube.
2) 2 µL aliquots of the 12M Hydrochloric Acid are added to the supernatant and inverted until a pH 2-3 was achieved.
3) All samples are vortexed for approximately 10 seconds.
4) 2 µL aliquots are taken from each sample to test on pH paper to ensure they had a pH of 2-3.
5) Samples are centrifuged for 5 minutes at 15000 rpm.
6) The supernatant transferred into 2 mL Eppendorf tubes and the cell pellet was discarded.
7) 900 µL of ethyl acetate is added to the 2mL Eppendorf tubes and vortexed for approximately 10 seconds.
8) Samples are centrifuged for 2.5 minutes at 15000rpm to ensure a good phase separation.
9) Transfer 800 µL of the ethyl acetate layer into 10mL glass test tubes.
10) Repeat steps 8-10 two more times. Note: Adjust the amount of Ethyl Acetate being added and removed according to volume available in the 2mL Eppendorf tube. Remaining water in the glass test tubes was removed using anhydrous magnesium sulfate.