Solid Phase Extraction (SPE) Microelution 96-Well Plates

General Methods and Support

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Sample Pretreatment

Plasma/Serum

Plasma and serum pretreatments are analyte dependent. If the analyte of interest is an acid, 2% phosphoric acid can be used (20µL 85% H_3PO_4 to 1mL of plasma or serum) to disrupt the drug-protein interaction. If the analyte of interest is basic, 0.1M sodium hydroxide can be used to disrupt the drug-protein interaction. After addition of acid or base, the sample should be vortexed for 20-30 seconds followed by centrifugation. The supernatant is now ready for further analysis.

Whole Blood

There are several pretreatment strategies that can be followed for whole blood. If the target analyte is present in red blood cells, a hemolysis step is necessary.

a. Hemolysis: To 0.2mL whole blood (spiked with analytes and internal standard) in a 1.2mL centrifuge tube, add 400µL of 2% zinc sulfate/80% methanol. Vortex for 10-20 seconds followed by centrifugation at 14,000 rpm for 10 minutes. Collect the supernatant for further analysis.

Preparation of zinc sulfate/methanol: Into a 100mL volumetric flask add 20mL water and 3.6g $ZnSO_4.7H_2O$. After the solution is clear and the salt crystals have dissolved, add 100% methanol. Refrigerate the solution at 2-8°C for 7 days.

b. Osmotic breakdown: To 1mL of whole blood add internal standard and 4mL of distilled water. Mix/vortex and let stand for 5 minutes. Centrifuge at 670g for 10 minutes and discard the pellet. Adjust the pH of the supernatant accordingly with the addition of a buffer solution.

c. Sonication: Sonicate 1mL whole blood for 15 minutes at room temperature. Add 3-6mL of an appropriate pH buffer (such as potassium phosphate buffer). Mix/vortex. Let stand for 5 minutes.

Urine

Enzymatic hydrolysis is necessary in case of conjugated forms (sulfated or glucuronide form) of the analye present. Enzymatic hydrolysis requires specific pH (pH 4-5) and temperature ranges. An acid or base hydrolysis can be performed as well, depending on the stability of the compound.

a. Enzymatic hydrolysis: To 500µL sample (spiked with analyte and internal standard) add 100µL acidic buffer (see below) and 20µL beta-glucuronidase. Vortex 5-6 seconds. Incubate in a water bath at 63°C for 30 minutes. Transfer sample to a 96-well collection plate or autosampler vial. Seal and centrifuge for 10 minutes at 2,000 rpm.

Preparation of acidic buffer (1.0M acetate buffer, pH 4.0): Dissolve 3.0g of glacial acetic acid and 4.1g of sodium acetate in a 1L volumetric flask.

b. Base hydrolysis: To 1mL urine (spiked with analyte and internal standard) add 100µL 10 N KOH. Mix, vortex, and hydrolyze for 20 minutes at 60°C. Cool and adjust pH to 3.5- 4.0 (by adding 200µL glacial acetic acid).

c. Acid hydrolysis: To 1mL urine add 0.25mL HCl in a screw capped test tube. Screw the tube top on loosely and heat in a boiling water bath for 60 minutes. Adjust to pH 7 (or as needed) with 1.0 N NaOH.

Saliva

No hydrolysis is required for oral fluids and the generic protocol used for plasma/serum pretreatment may be followed.

Tissue

Homogenize with organic or aqueous solvent depending upon analyte solubility. Settle, decant, centrifuge or filter supernatant. Perform direct Matrix Solid Phase Dispersion (MSPD) extraction on tissue.

Strata®-X Sorbent Selection and General Methods

	Target Analyte		Target Analyte	Target Analyte	
	Acidic Compounds		Neutral Compounds	Basic Compounds	
	pK_a <2	pK_a 2-4	Neutral/Uncharged	pK_a 8-10	pK_a >10
	Strata-X-AW	Strata-X-A	Strata-X	Strata-X-C	Strata-X-CW
Condition	200 µL Methanol	200 µL Methanol	200 µL Methanol	200 µL Methanol	200 µL Methanol
Equilibrate	200 µL Water (pH 6-7)	200 µL Water (pH 6-7)	200 µL Water	200 µL Acidified Water	200 µL Water (pH 6-7)
Load	25-750 µL pretreated sample	25-750 µL pretreated sample	25-750 µL pretreated sample	25-750 µL pretreated sample	25-750 µL pretreated sample
Wash 1	200 µL 25 mM Ammonium Acetate (pH 6-7)	200 µL 25 mM Ammonium Acetate (pH 6-7)	200 µL 5-60 % Methanol	200 µL 0.1 M HCl in Water	200 µL Water (pH 6-7)
Wash 2	200 µL Methanol	200 µL Methanol		200 µL 0.1 M HCl in Methanol	200 µL Methanol
Elute*	Weak Acids: 25 µL 5 % Formic Acid in Methanol. Wait 1 minute then apply 5˝ Hg vacuum to complete elution and continue 1 minute thereafter. Any Acid: 25 µL 5 % NH_4OH in Methanol. Wait 1 minute then apply 5˝ Hg vacuum to complete elution and continue 1 minute thereafter.	25 µL 5 % Formic Acid in Methanol. Wait 1 minute then apply 5˝ Hg vacuum to complete elution and continue 1 minute thereafter.	25 µL 2 % Formic Acid in Methanol/ Acetonitrile. Wait 1 minute then apply 5˝ Hg vacuum to complete elution and continue 1 minute thereafter.	25 µL 5 % NH_4OH in Methanol. Wait 1 minute then apply 5˝ Hg vacuum to complete elution and continue 1 minute thereafter.	Weak Bases: 25 µL 5 % NH_4OH in Methanol. Wait 1 minute then apply 5˝ Hg vacuum to complete elution and continue 1 minute thereafter. Any Base: 25 µL 5 % Formic Acid in Methanol. Wait 1 minute then apply 5˝ Hg vacuum to complete elution and continue 1 minute thereafter.

No Dry Down or Reconstitution Step Required

* It may be possible to boost recoveries of target analytes by eluting in a larger volume such as 2x 25 µL, however this will result in a more dilute sample.

Easily Process Samples

Strata®-X microelution 96-well plates are compatible with standard vacuum manifolds, positive pressure manifolds, and liquid handling systems, making it even easier to process your samples.

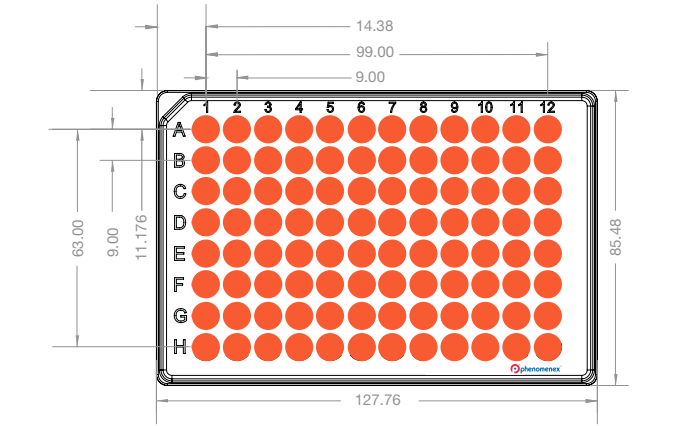
Cost Effective Benchtop Processing

96-Well Plate
Vacuum Manifold
Part No. AH0-8950

Presston™ 100
Positive Pressure Manifold
Part No. 9334

High-Throughput, Hands Free Liquid Handling Systems

Use the dimensions outlined below to program your liquid handling system.



measured in mm

Don’t Forget Collection Plates and Sealing Mats

Collection Plates (deep well, polypropylene)

Part No.	Description	Unit
AH0-7192	350 µL/well, Square	50/pk
AH0-7193	1 mL/well, Square	50/pk
AH0-7279	1 mL/well, Round/Round Bottom, 7 mm	50/pk
AH0-7194	2 mL/well, Square	50/pk
AH0-8635	2 mL/well, Square/Round-Conical Bottom	50/pk
AH0-8636	2 mL/well, Round/Round Bottom, 8 mm	50/pk

Sealing Mats

Part No.	Description	Unit
AH0-8633**	Sealing Mats, Pierceable, 96-Round Well 8 mm, Silicone	50/pk

** 8 mm round-well sealing mats compatible with Strata®-X microelution 96-Well Plates and 2 mL round-well 8 mm collection plates (AH0-8636)

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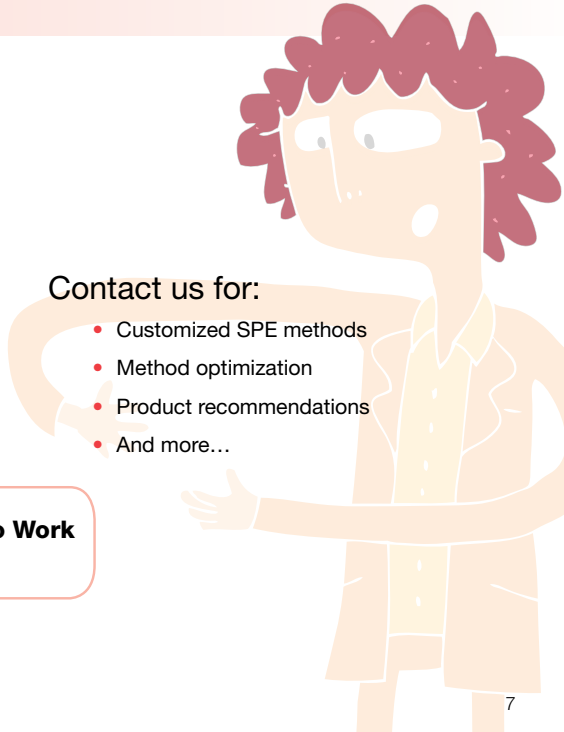

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