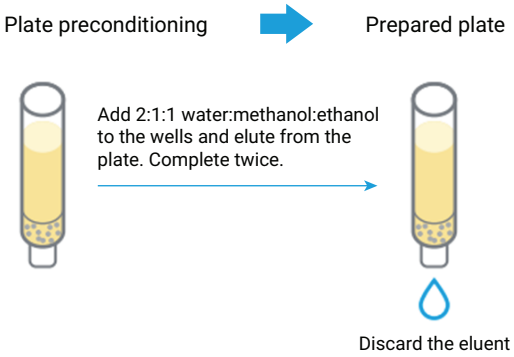
Agilent Captiva EMR–Lipid Automated Plate Preconditioning

Method guide for 96-well plates

General information

LC/MS omics sample preparation methods[1-3] that use Agilent Captiva EMR–Lipid technology can benefit from plate preconditioning. This method guide describes an automated plate preconditioning process that uses a supplementary Agilent VWorks protocol designed for the Agilent Bravo Metabolomics Sample Prep Platform (Metabolomics Bravo). After plate preconditioning, metabolites, lipids, and proteins can be fractionated from plasma and mammalian cell samples using automated sample preparation methods[1-3] developed for Agilent end-to-end LC/MS omics workflows.



Protocol

Solvent solution preparation for a 96-well plate

Prepare solvent solution.

1. Have 180 mL of 2:1:1 water:methanol:ethanol (preconditioning solvent solution) ready for use.

   Note: Make the preconditioning solvent solution in batches of 0.4 to 1 L and use the solvent solution within 3 to 4 months.

   Note: Use LC/MS-grade or ultrahigh purity solvents.

   Note: The preconditioning solvent solution composition should approximate the composition of the sample when passed through the Captiva–EMR Lipid plate and can be adjusted as necessary.

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Automated Captiva EMR–Lipid 96-well plate preconditioning protocol

1. Open the plate preconditioning form file on the Bravo Metabolomics Sample Prep Platform software.

   Note: Set the number of columns to be preconditioned and the starting column.

   Note: Seal any wells of the Captiva EMR–Lipid plate not being preconditioned with a plate seal or lab tape.

2. Place plates and the preconditioning solvent solution on the Metabolomics Bravo as shown in the protocol form and start the protocol.

3. The Metabolomics Bravo will sequentially transfer two 800 µL aliquots of the preconditioning solvent solution to each well of the Captiva EMR–Lipid plate that is being preconditioned.

4. The Metabolomics Bravo will sequentially pull each aliquot of the preconditioning solvent through the wells of the Captiva EMR–Lipid plate under vacuum.

   Note: The **Manually confirm filtration is complete** box in the VWorks protocol form can be checked to ensure that all wells of the Captiva EMR–Lipid plate have been emptied. Additional filtration time and pressure can be added until all wells are empty.

Materials

- Ultrapure water produced with a Milli-Q Integral System equipped with an LC-Pak Polisher and a 0.22 µm point-of-use membrane filter cartridge (MilliporeSigma)
- LC/MS-grade methanol
- High purity > 99.5% ethanol

Agilent hardware and consumables

Description	Quantity	Part Number
Bravo Metabolomics Sample Prep Platform	Each	G5589AA
Agilent Captiva EMR—Lipid plates	5 plates	5190-1001
Agilent 250 µL disposable tips, 96 in rack	50 racks	19477-002
Agilent reservoir, single cavity	25 plates	201244-100

References

1. Sartain, M.; *et al.* Enabling Automated, Low-Volume Plasma Metabolite Extraction with the Agilent Bravo Platform. *Agilent Technologies application note*, publication number 5994-2156EN, **2020**.
2. Van de Bittner, G. C.; *et al.* An Automated Dual Metabolite + Lipid Sample Preparation Workflow for Mammalian Cell Samples. *Agilent Technologies technical overview*, publication number 5994-5065EN, **2022**.
3. Van de Bittner, G .C.; *et al.* Automated Fractionation of Low-Volume Plasma Samples for LC/MS Multi-Omics. *Agilent Technologies technical overview*, publication number 5994-7357EN, **2024**.

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