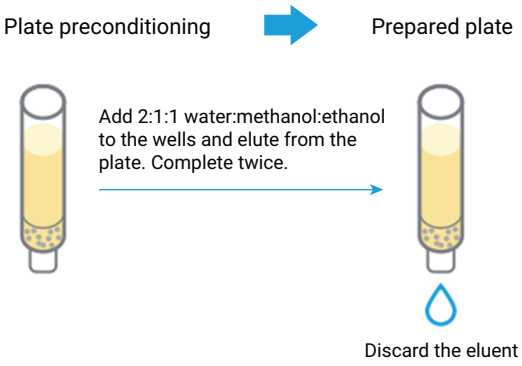
Agilent Captiva EMR–Lipid Manual Plate Preconditioning

Method guide for 96-well plates

General information

LC/MS omics sample preparation methods[1-4] that use the Agilent Captiva EMR–Lipid technology can benefit from plate preconditioning. This method guide describes a manual plate preconditioning process that uses a positive pressure manifold. After plate preconditioning, metabolites, lipids, and proteins can be fractionated from plasma and mammalian cell samples using manual or automated sample preparation methods[1-4] 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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Manual Captiva EMR–Lipid 96-well plate preconditioning protocol

1. Add 800 µL of the preconditioning solvent solution to each well of a Captiva EMR–Lipid plate that is being preconditioned.

   Note: Seal any wells not being preconditioned with a plate seal or lab tape.

   Note: The preconditioning solvent solution can be transferred from a single well reservoir to a Captiva EMR–Lipid plate using a multichannel pipettor.

2. Use a positive pressure manifold, such as an Agilent PPM-96, to push the preconditioning solvent solution through the plate wells.

   Note: Use a 2-column reservoir to collect the preconditioning solvent solution eluent.

   Note: The Agilent PPM-96 low flow setting is recommended. Gradually increase the pressure until the preconditioning solvent solution flows through the plate wells.

3. Add a second 800 µL aliquot of preconditioning solvent solution to each well of a Captiva EMR–Lipid plate that is being preconditioned.

4. Use a positive pressure manifold to push the preconditioning solvent solution through the plate wells.

   Note: After the plate wells have been emptied, the pressure or gas flow can be switched to the high flow setting, 10 to 20 psi, to release additional preconditioning solvent solution from the sorbent and filter frits.

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
Agilent Captiva EMR—Lipid plates	5 plates	5190-1001
Agilent reservoir, 2-column	25 plates	203852-100
Agilent reservoir, single cavity	25 plates	201244-100
Positive pressure manifold 96 processor for plates	Each	5191-4116

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 and 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**.
4. Agilent Captiva EMR–Lipid Manual Fractionation of Low-Volume Plasma Samples for LC/MS Multi-Omics. *Agilent Technologies method guide*, publication number 5994-7482EN, **2024**.

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