Agilent Captiva EMR–Lipid Manual Fractionation of Low-Volume Plasma Samples for LC/MS Multi-Omics

Method guide for 96-well plates

General information

Manual multi-omics sample preparation dramatically benefits from a consistent, robust, and easy-to-implement methodology that is readily automated when sample throughput or reproducibility requirements necessitate the transition to automation. This method guide describes a manual 20 µL plasma sample preparation method that was tested for automation performance with an Agilent Bravo Metabolomics Sample Prep Platform.[1] The method has multiple configurations, which enable the user to select which combination of metabolite, lipid, and protein fraction(s) to collect and optimize to meet the requirements of a particular LC/MS omics study.

To begin, first determine whether to collect a protein fraction, based on the decision tree below. After making this selection, the workflow steps outlined in the following pages are followed until all desired sample fractions have been collected. As with previously described methods[2,3], this protocol uses Agilent Captiva EMR–Lipid plates to enable fractionation of polar metabolites and lipids. If collected, protein pellets can be further prepared according to common LC/MS protein preparation protocols.[4] All collected plasma fractions are suitable for LC/MS analysis and have been tested using Agilent metabolite[5], lipid[6], and peptide[7] LC/MS methods. Notably, a LC/TQ or LC/Q-TOF instrument can be adapted to complete analysis of all three sample fractions, enabling a quick turnaround for end-to-end multi-omics analyses.[8]

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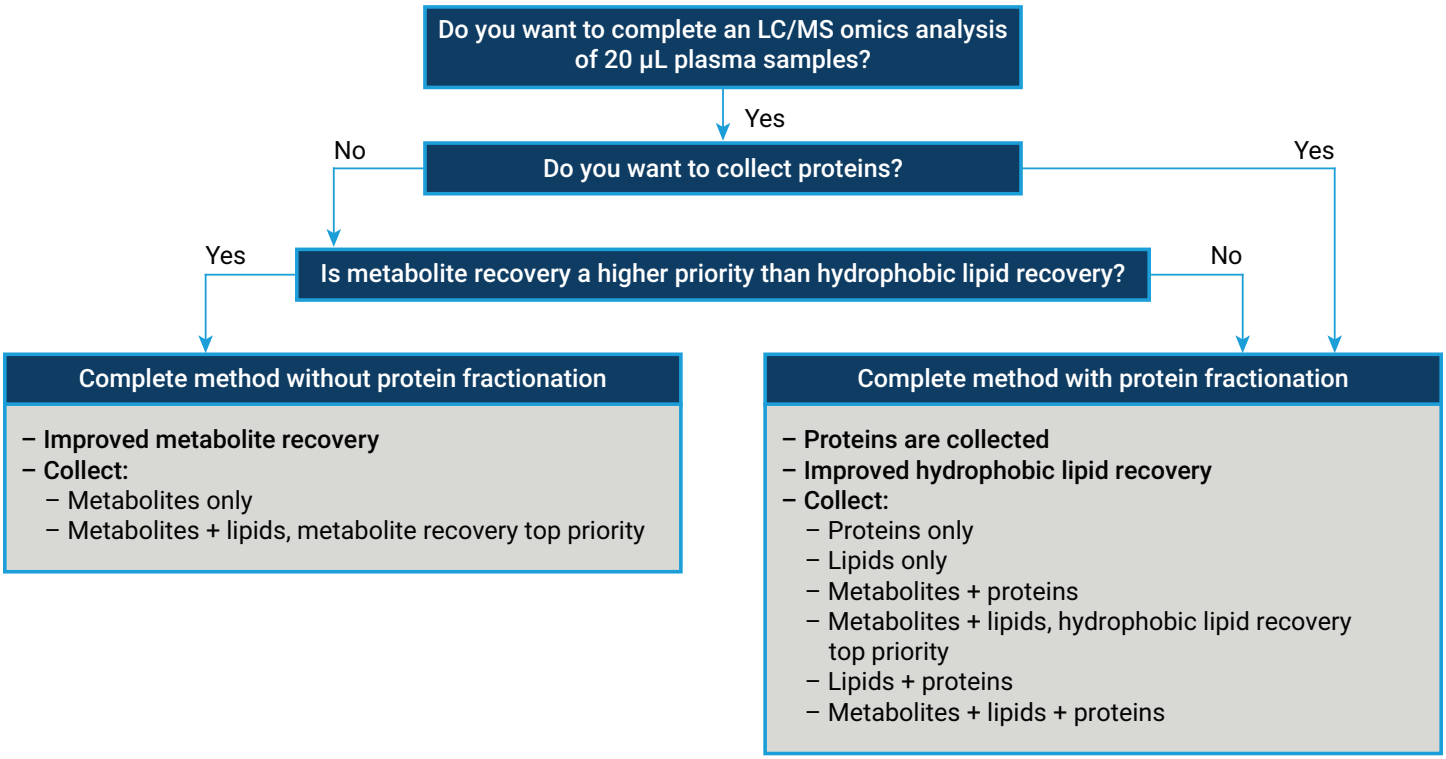
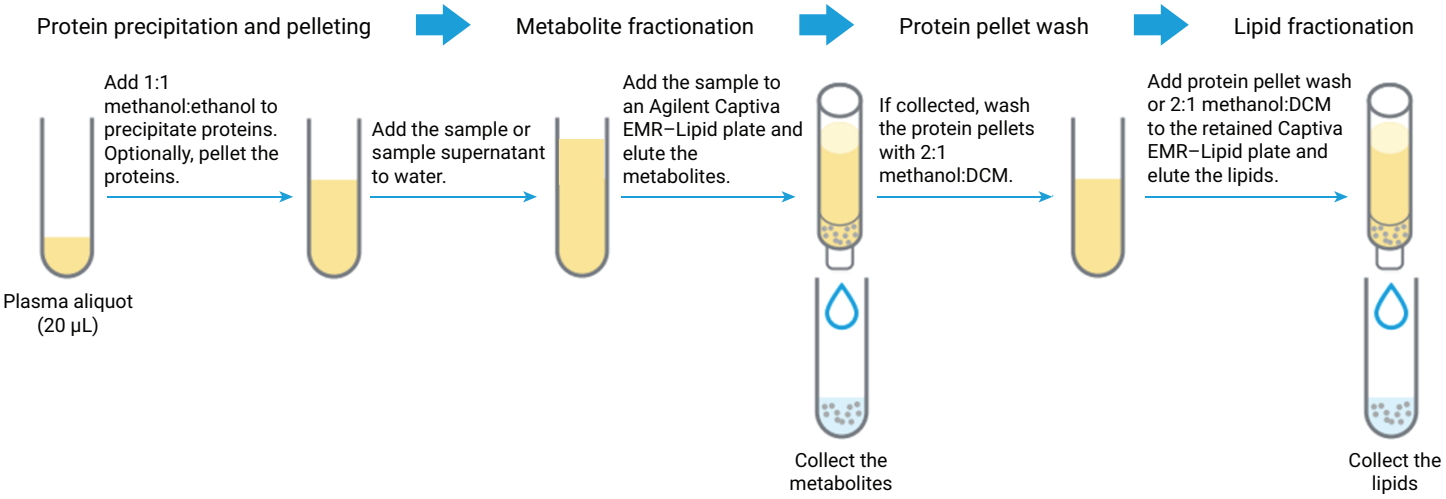
Trusted Answers


Do you want to complete an LC/MS omics analysis of 20 µL plasma samples?
Yes
Do you want to collect proteins?
No
Yes
Is metabolite recovery a higher priority than hydrophobic lipid recovery?
Yes
No
Complete method without protein fractionation
– Improved metabolite recovery
– Collect:
– Metabolites only
– Metabolites + lipids, metabolite recovery top priority
Complete method with protein fractionation
– Proteins are collected
– Improved hydrophobic lipid recovery
– Collect:
– Proteins only
– Lipids only
– Metabolites + proteins
– Metabolites + lipids, hydrophobic lipid recovery top priority
– Lipids + proteins
– Metabolites + lipids + proteins


Protein precipitation and pelleting
Metabolite fractionation
Protein pellet wash
Lipid fractionation
Add 1:1 methanol:ethanol to precipitate proteins. Optionally, pellet the proteins.
Add the sample or sample supernatant to water.
Add the sample to an Agilent Captiva EMR–Lipid plate and elute the metabolites.
If collected, wash the protein pellets with 2:1 methanol:DCM.
Add protein pellet wash or 2:1 methanol:DCM to the retained Captiva EMR–Lipid plate and elute the lipids.
Plasma aliquot (20 µL)
Collect the metabolites
Collect the lipids

Protocol

Solvent solution preparation for a 96-well plate

Prepare solvent solutions.

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

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

1. Have 20 mL of Milli-Q water ready for use.
2. Have 20 mL of 1:1 (v:v) methanol:ethanol ready for use.
3. Have 80 mL of 2:1:1 (v:v:v) water:methanol:ethanol ready for use.
4. Have 200 mL of 2:1 (v:v) methanol:dichloromethane ready for use.

Metabolite, lipid, and protein fractionation

1. Place 20 µL plasma samples into wells of a 96-well plate.

   Note: Do not transfer plasma samples directly to the Captiva EMR–Lipid plate.

   Note: If lipids are being collected using a protein fractionation protocol configuration, the plasma samples must be added to a glass-lined plate to ensure compatibility with the protein pellet wash solution in step 9.

   a. Centrifuge plate at 250 × g for 30 seconds to pull plasma samples to the bottom of the plate.

2. Transfer 112.5 µL of room temperature 1:1 methanol:ethanol to each plasma sample.

   Note: Internal standards are often included in the 1:1 methanol:ethanol solution.

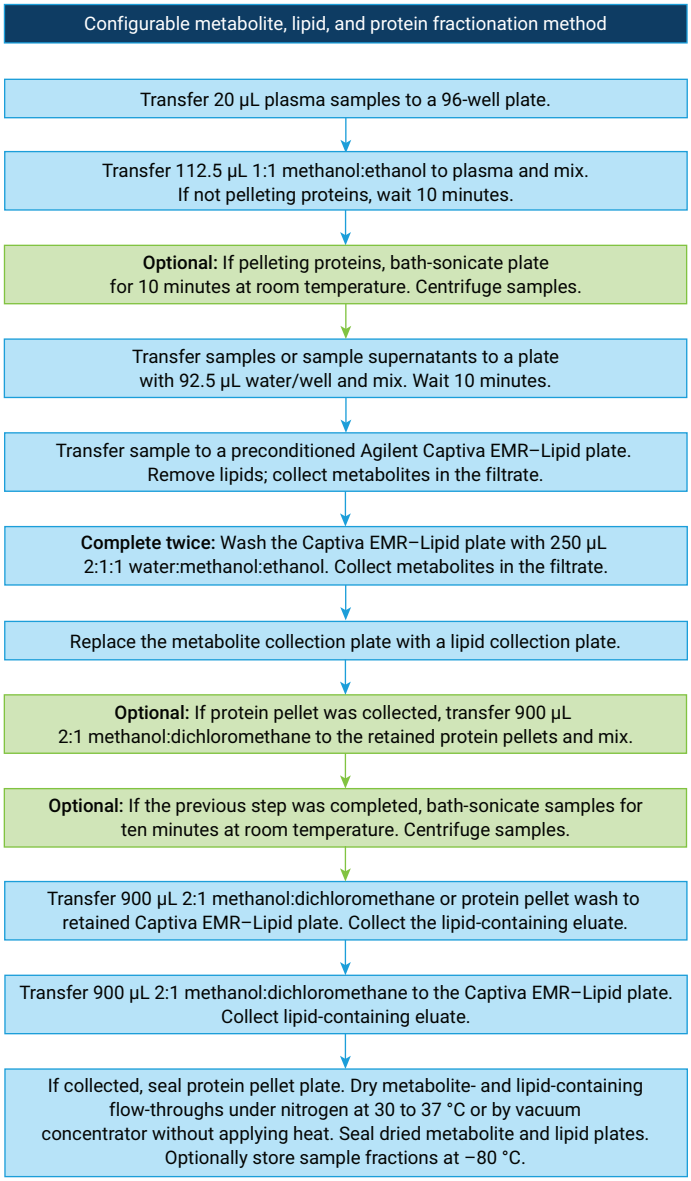
   a. Pipette up and down 2 to 4 times to mix.

   b. Optionally mix with a vortex mixer for 5 to 10 seconds.

3. If not pelleting proteins, wait 10 minutes for sample equilibration.
4. Optional protein fractionation: Bath-sonicate the sample plate for 10 minutes at room temperature.

   a. Centrifuge the plate for 60 minutes at 2,272 × g and 20 °C to pellet the protein precipitates.

   Note: Given the low centrifuge speeds used for plate centrifugation, a long centrifugation is required for protein pelleting.



5. Transfer the samples or sample supernatants to a plate containing 92.5 µL of water per well.

   Note: The plate containing water, to which the samples or sample supernatants are transferred in this step, is the sample plate referred to in step 8.

   a. Pipette up and down 2 to 4 times to mix.

   b. Optionally mix water-containing samples with a vortex mixer for 5 to 10 seconds.

   c. If collected, retain protein pellets in the original glass-lined plate.

6. Wait 10 minutes for sample equilibration.
7. Transfer the samples to a preconditioned[9] Captiva EMR–Lipid plate.
    a. Pass the samples through the plate and into a metabolite collection plate using a positive pressure manifold processor such as the Agilent PPM-96. The low flow setting is recommended. The target flow rate through the plate is 1 drop every 1 to 2 seconds.

       Note: It is recommended to gradually increase the pressure until the samples flow through the plate wells at the desired flow rate.

    b. After filtration appears complete, increase the pressure for 1 minute to elute additional sample solution retained by the sorbent and filter frits.
8. Wash the wells of the Captiva EMR–Lipid plate twice with 250 µL of 2:1:1 water:methanol:ethanol following the positive pressure filtration instructions outlined in the previous step.

       Note: The 2:1:1 water:methanol:ethanol wash solvent can be used to wash the sample plate from step 5 prior to adding the wash solvent to the Captiva EMR–Lipid plate.

    a. Collect the eluent into the same metabolite collection plate used previously.
9. Remove and temporarily seal the metabolite collection plate with a nonadhesive, heat-sealed Agilent PlateLoc seal and centrifuge the plate at 250 × g for 20 seconds to pull down any liquid on the walls of the plate.
    a. Add a glass-lined lipid collection plate below the Captiva EMR–Lipid plate.
10. If proteins were fractionated: Transfer 900 µL of 2:1 methanol:dichloromethane to the retained protein pellets in the original glass-lined plasma sample plate and pipette up and down 2 to 4 times.

       Note: This protein pellet wash step is crucial for obtaining maximum lipid recovery when the protein pellet is collected.

    a. Bath-sonicate sample plate for 10 minutes at room temperature.

       Note: Lipid recovery will be maximized even for samples in which the protein pellet is not fully resuspended during sonication.

    b. Centrifuge the plate for 60 minutes at 2,272 × g and 20 °C to re-pellet the protein precipitate.
11. Transfer 900 µL of 2:1 methanol:dichloromethane or the protein pellet wash to the retained Captiva EMR–Lipid plate.

       Note: Transfer the solvent to the Captiva EMR–Lipid plate only after the glass-lined lipid collection plate is in place below the Captiva EMR–Lipid plate.

    a. Pass the solution through the Captiva EMR–Lipid plate and into the lipid collection plate using a positive pressure manifold processor such as the PPM-96. The low flow setting is recommended. The target flow rate through the plate is one drop every 3 to 5 seconds.

       Note: It is recommended to gradually increase the pressure until the samples flow through the plate wells at the desired flow rate.

       Note: A slow elution rate is key to maximizing lipid elution.

    b. After filtration appears complete, increase the pressure for 1 minute to elute additional sample solution held up by the sorbent and filter frits.
12. Transfer a second 900 µL aliquot of 2:1 methanol:dichloromethane to the retained Captiva EMR–Lipid plate.
    a. Follow the positive pressure filtration instructions outlined in the previous step.
    b. Collect the lipid-containing eluent into the same glass-lined plate used previously.
    c. Remove the lipid collection plate.

Sample drying and storage

1. Dry the metabolite and lipid samples under nitrogen at 30 to 37 °C or by vacuum concentrator without applying heat.

   Note: For nitrogen drying, use of high nitrogen flow that is continuously at a close distance to the top of the sample reduces drying time. Decrease nitrogen flow when samples are reduced to less than 50 µL to prevent ejection of dried samples from the plate.

   Note: Samples should be removed from nitrogen flow as soon as they are dry.

   Note: Protein pellets do not need to be dried.
2. If sample fractions are being stored prior to protein preparation or LC/MS analysis, seal the sample fraction plate(s) with a nonadhesive, heat-sealed PlateLoc seal, and store the samples at –80 °C.

Metabolite and lipid sample reconstitution

1. Reconstitute the samples in 50 to 200 µL of a suitable LC/MS solvent.
   a. Seal the plate with a nonadhesive, heat-sealed, pierceable PlateLoc seal.
   b. Samples can be bath-sonicated, shaken with an orbital shaker, and/or mixed through vortex to assist with redissolution.
2. Centrifuge the samples for 30 seconds at 250 × g to bring all liquid to the bottom of the plate.

   Note: Some LC/MS solvents may not fully redissolve the metabolite sample. In this case, the samples can be centrifuged at maximum rpm for 3 minutes and the supernatants can be transferred to low volume LC/MS vials or a 96-well plate sealed with a nonadhesive, heat-sealed, pierceable PlateLoc seal.

Protein pellet preparation

1. Protein pellets can be prepared according to common LC/MS protein preparation protocols.[4]

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
- GC/MS-grade dichloromethane (DCM)

Instruments and consumables

Description	Quantity	Part Number
Agilent Captiva EMR—Lipid plates	5 plates	5190-1001
Agilent Captiva collection plates	10 plates	A696001000
Agilent reservoir, single cavity	25 plates	201244-100
Thermo Fisher Scientific SureSTART WebSeal Plate+ 96-well deep well plates	10 plates	60180-P338
Positive pressure manifold 96 processor for plates	Each	5191-4116
Agilent PlateLoc thermal microplate sealer	Each	G5585BA
Agilent PlateLoc microplate clear pierceable thin seal	Roll	17318-001

References

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