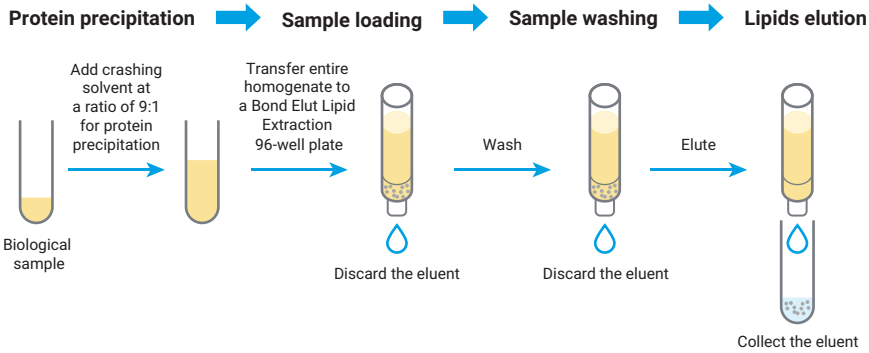
Agilent Bond Elut Lipid Extraction

Method guide for 96-well plate

General instructions

Agilent Bond Elut Lipid Extraction 96-well plates allow the extraction and isolation of lipids from biological samples such as plasma, cell cultures, and tissue samples. The unique EMR—Lipid sorbent chemistry provides effective and selective retention of lipid compounds on the sorbent. After a washing step, the trapped lipid compounds are eluted with solvent. The 96-well plate SPE format simplifies the extraction process compared to traditional liquid-liquid extraction techniques used for lipid analysis. The workflow provides significant time savings with equivalent extraction efficiency and improved method reproducibility. Bond Elut Lipid Extraction 96-well plates are suitable for high-throughput sample preparation, and are easily automatable.



1. **Crashing solvent:** ACN with 5% MeOH is recommended. A small percentage of MeOH helps to generate finer protein precipitates, which allows for easy pipette transfer of the homogenate.
2. **Washing solvent:** a mixture of ACN with 10 to 20% of water.
3. **Elution solvent:** a solution of MeOH with dichloromethane (DCM), chloroform, or 1-chlorobutane. A minimum of 50% MeOH is important for the release of lipids. Agilent recommends DCM/MeOH (v/v 1:2) or chloroform/MeOH (v/v 1:1).

For more information, visit:

www.agilent.com/chem/BondElutLE

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A recommended Bond Elut Lipid Extraction protocol for plasma extraction

1. Add 100 µL of plasma into each well of a 2 mL collection plate.
2. Add 900 µL of ACN with 5% MeOH, cover the plate with a plate mat, and sonicate on ice for 10 minutes.
3. Transfer the entire homogenate to a Bond Elut Lipid Extraction 96-well plate using a multichannel pipette or liquid handler.
4. Process with gravity elution. Discard the eluent.
5. Add 2 × 1 mL of ACN/water (v/v 9:1) to the previous collection plate for rinsing, and then transfer to the Bond Elut Lipid Extraction 96-well plate. Use gravity for elution, and discard the eluent.
6. Apply higher vacuum or positive pressure at the end to completely dry the plate. Place an appropriate collection plate beneath the Bond Elut Lipid Extraction 96-well plate.
7. Add 1 mL of DCM/MeOH (v/v 1:2) for elution and collect the eluent.
8. Carefully lift the Bond Elut Lipid Extraction 96-well plate and ensure that there is no eluent dripping from the tips. Place the Bond Elut Lipid Extraction plate in a clean location. Dry the eluent in the collection plate with N_2 at 30 °C for 10 to 15 minutes.
9. Place the same dried collection plate back under the Bond Elut Lipid Extraction 96-well plate.
10. Add another 1 mL of DCM/MeOH (v/v 1:2) for gravity elution and collect the eluent. When there is no visible liquid left in the wells, apply high vacuum or pressure to dry the sorbent bed.
11. Dry the eluent with N_2 at 30 °C.
12. Reconstitute into 100 µL of *n*-BuOH/MeOH (v/v 1:1), and cover the plate.
13. Vortex for two minutes, sonicate for 10 minutes, then centrifuge for five minutes. Samples are then ready for MS analysis.

Product use tips and tricks

1. A single well waste plate (p/n 5191-4121 or similar) can be used with the Bond Elut Lipid Extraction 96-well plate for steps 1 to 5.
2. Gravity elution should be feasible for sample loading, washing, and elution steps. Occasionally, external force such as vacuum or positive pressure is needed to assist the elution. The ideal flow rate is 3 to 5 seconds per drop.
3. Lipids can be trapped in the protein precipitate. It is important to transfer the entire homogenate in Step 3. **Do not centrifuge** before transferring.
4. Wide-bore pipette tips are recommended for transfer. Pipetting premixing is necessary before transfer (Step 3).
5. The collection plate and plate mat should be chemically resistant to elution solvents such as DCM and chloroform. This prevents sample contamination with plastic leachables. A collection plate with glass insert or quartz-coated polypropylene plate should be used. The well volume should be 1.2 mL or above to collect the eluent appropriately without cross-contamination.

Bond Elut Lipid Extraction ordering information

Description	Quantity	Part Number
Agilent Bond Elut Lipid Extraction, 1 mL cartridge	100/pk	5610-2041
Agilent Bond Elut Lipid Extraction, 96-well plate	1 plate	5610-2042
Agilent Bond Elut Lipid Extraction, 96-well plate	5 plates	5610-2043

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