Frequently Asked Questions About Baulo™ PLE

1. **General**

1) Why we use PPP/PPT:

Fast-flow PP is a revolutionized sample preparation technique. Baulo™ PLE PPT/PPP are developed based on specially selected membrane and virgin plastic materials. The optimized filtration systems provide an automated solution for efficient removal of protein and lipids. With PPT/PPP, the whole analysis procedure can be streamlined to save both time、reagents and costs.

2) What kind of results can I expect from PP process?

Generally, sensitivities obtained from Baulo™ PLE processed samples are better than regular PP alone. Specific enhancements of sensitivity will depend on the analyte susceptibility to ion suppression, the quality of the HPLC separation of the analyte from the ion suppressing lipids, and the amount of lipids built-up on the column. Routine use of Baulo™ P&LE can minimize the carry-over of the column.

3) How do I know I am removing all the lipids?

The easiest way to monitor the ion suppressing phospholipids during method development is to monitor the phosphatidylcholine head group with a 184g184 m/z trace (in-source fragmentation). Lysophosphatidylcholines, responsible for the lipid ion-suppression front seen in a single injection, may elute in the high organic section of typical reverse phase gradient programs. In the high throughput environment, diacylphosphatidylcholines typically carry over to subsequent injections and are responsible for long-term build-up and additional ion suppression.

2. Addition of samples and crash solvents

1) What is the limits of sample volumes?

For PPT, the specific sample volumes will depend on the sizes of the tubes. For PPP, the suggested plasma sample volume is between 50 and 200 µL based on lipid capacity and the ease of in-well processing. Larger sample volumes may not fit in the well after dilution. These samples can be precipitated in an appropriate container and filtered through the plate separately. Key to processing smaller sample volumes is complete coverage of the bottom frit. Sample volumes below 50 µL should be pre-diluted with aqueous and the overall organic ratio maintained.

2) What organic crash solvents can I use?

Baulo™ PLE is designed to remove the lipid interferences from both acetonitrile and methanol-based precipitations. Solvent modifications can be optimized to more fully remove either proteins or lipids. The following general guidelines should be followed:

A. **Best General Method:** 3:1 with pH Modified MeOH；
B. **Optimized for Protein Removal**：3:1 or more with pH Modified CAN when a vacuum of 250 to 400 mm of Hg is used. If positive pressure mode is used, make sure to thoroughly mix and precipitate before applying the pressure.
C. **Optimized for Lipid Removal**： 2:1 with pH Modified MeOH

3) *How to modify pH?*

Modifying pH conditions to ionize the analyte of interest will help avoid undesired interactions and increase the compatibilities. This is especially true for hydrophobic side compounds even though polar analytes may less affected by modification. Therefore, the choice of pH modifier and pH condition should be related to the Log D value of the analyte.

4) *Can I change the order of adding the sample/crash solvent addition?*

The order of addition usually does not prevent the process of running. However, the order of addition does affect the results, and a single order is recommended for any one assay.

3. Precipitation Stage

1) *Do I have to mix?*

Samples should be mixed thoroughly to precipitate. If plasma proteins are not denatured prior to filtration, they can precipitate in the frit, the membrane or beyond, leading to irreproducible flow, clogging, or cloudy filtrates.

2) *What other mixing options do I have?*

Pipette mixing is sufficient to shear particulate droplets and denature the plasma proteins fully. However, most orbital mixers merely swirl the sample and do not mix top-to-bottom well enough.

Certain plasmas and crash solvent combinations may precipitate better than others and minimize the need for mixing. Please verify flow and filtrate clarity on your sample prior to proceeding on any non-mixing solution.

4. Filteration Stage

1) *Why is the recovered volume is lower?*

The precipitation and removal of proteins reduces the volume. Filter cakes should be dried sufficiently to avoid additional losses.

2) *Can I evaporate and reconstitute?*

Baulo™ PLE is designed to facilitate direct injection of filtrates and improve the evaporation and reconstitution process. It depletes protein and lipid and allows you to concentrate the analyte in small volumes without increasing the concentration of protein or lipid interferences.