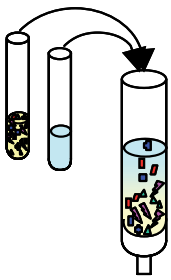
Captiva™ ND*Lipids*

NON-DRIP FILTRATION PLATE FOR LIPID & PROTEIN DEPLETION

Captiva ND*Lipids* is a simple to use filtration device designed for the removal of lipids and protein interferences from protein precipitated plasma and serum samples.
Phospholipid carry-over is one of the main problems in LC/MS-(MS) bioanalysis when using protein precipitation as the sample preparation technique.
Lipids that elute during the HPLC run can cause ion suppression and limit analyte sensitivity and detection. Over the course of multiple injections, lipids build up and bleed off the column, causing ion suppression, shifts in retention time and peak shape and necessitating additional column and system maintenance. Lipid and protein removal is especially important in a high throughput environment where short run times and ballistic gradients are insufficient to deal with these interferences chromatographically.
Use of Captiva ND*Lipids* will virtually eliminate these effects and extend the lifetime of your analytical column.
This user guide describes how to efficiently use the Captiva ND*Lipids* phospholipid and protein filtration plate.

Easy 3-Step Procedure

1 Add



Add plasma and crash solvent in desired ratio

Add Plasma:

- 50-200 µL Plasma

Add Crash Solvent:

- For optimal lipid and protein removal, 3:1 methanol to plasma ratios are recommended
- pH modification can be made to ionize the analyte of interest
- For basic compounds, we recommend 0.1% to 1.0% formic acid in methanol
- For acidic compounds, we recommend 5 mM to 10 mM ammonium formate buffers at pH 9
- Captiva ND*Lipids* can be used with various organic crash solvents, modifiers and ratios. Refer to FAQ's for details.
- Addition order can be altered according to user preference

2 Precipitate

For thorough precipitation, pipette mixing is recommended

Mixing:

- 3 to 5 pipette strokes of 3/4 combined liquid volume is sufficient to thoroughly precipitate plasma proteins
- Orbital/vortex mixing of the 96 well plate is insufficient to thoroughly precipitate plasma proteins in methanol
- See the FAQs for additional details

3 Filter

Salts
Proteins
Lipids
Analyte

Pass sample through filter and collect filtrate

Flow rate:

- Flow rate is highly dependent on plasma type, age, and mixing
- Samples should pass in under 5 min depending on volume
- Unmixed or difficult (rat) plasmas may take longer to pass through the 0.2 µm filter

Volume collected:

- Recovery volumes should represent 75-85% of the combined liquid volumes added
- The majority of volume reduction comes from the precipitation and removal of proteins. Filter cakes should be dried sufficiently to avoid additional losses
- See the FAQs for additional details

Frequently Asked Questions

General

What kind of results can I expect?

Generally, sensitivities obtained from Captiva ND^{Lipids} processed samples are better than protein precipitation alone. Sensitivity enhancements 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 Captiva ND^{Lipids} can keep column carry-over to a minimum.

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 184→184 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.

Add

Is it possible to use larger or smaller sample volumes?

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.

What other organic crash solvents can I use?

Captiva ND^{Lipids} is designed to remove the lipid interferences from both acetonitrile and methanol-based precipitations. Solvent modifications can, however, be optimized to more fully remove either proteins or lipids. The following table gives an overview of crash solvent selection:

Optimized for Protein Removal	Best General Method	Optimized for Lipid Removal
3:1 or more with pH Modified ACN*	3:1 with pH Modified MeOH	2:1 with pH Modified MeOH

*Filtration mode used: Vacuum 254 to 381 mm of Hg. When using positive pressure manifold make sure to thoroughly mix and precipitate before applying the pressure.

Do I have to pH modify, and if so, what pH modifiers can I use?

The use of pH modified precipitation conditions to ionize the analyte of interest will help avoid undesired interactions with the plate, and will increase the range of analyte polarities compatible with the plate especially on the hydrophobic side (the log P cut-off value for analyte compatibility is approximately 5.5). Polar analytes may not need the modification.

The choice of pH modifier and pH condition should be based upon the analyte Log D profile.

Can I alter the sample/crash solvent addition order?

The non-drip feature of the Captiva ND^{Lipids} plate allows the plate to be used in either order of addition. Addition order may affect results, and a single order is recommended for any one assay.

Precipitate

Do I have to mix?

Thorough precipitation is the goal of thorough mixing. Plasma proteins not denatured prior to filtration can precipitate in the frit, the membrane or beyond, leading to irreproducible flow, clogging, or cloudy filtrates.

What other mixing options do I have?

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

Filter

Why is the filtrate volume lower than the mixture of sample and crash solvent?

The majority of volume reduction comes from the precipitation and removal of proteins. Filter cakes should be dried sufficiently to avoid additional losses.

Can I evaporate and reconstitute?

Captiva ND^{Lipids} is designed to facilitate direct injection of filtrates and improve the evaporation and reconstitution process. Protein and lipid depletion using Captiva ND^{Lipids} allows you to concentrate the analyte in small volumes without increasing the concentration of protein or lipid interferences.

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