Active Background Ion Reduction Device
What you need to know about ABird

ABIRD is a popular accessory for nanospray-powered mass spectrometers, and has been approved for use on all Thermo Scientific instruments and nanospray sources.

ABIRD will consistently:

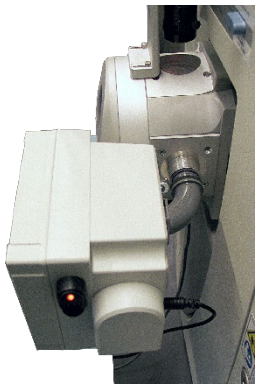
- Isolate the instrument from changing background ions, which is critical for extended quantitative or comparative analyses.
- Reduce background ion signals, enhancing S/N ratio quite dramatically in many cases.

These are benefits that only an ABIRD can provide, making it a smart enhancement for any nanospray ESI/LC-MS system.

Many user hope to demonstrate improved peptide detection with ABIRD installed, but get frustrated when they cannot easily do so. Given the improved signal to noise ratio ABIRD provides to a system, it might seem easy to find more peptides with ABIRD than without. We have found, however, that this is far from a simple task, and that there are many factors that need to be optimized in order to be able to demonstrate this in your own laboratory.

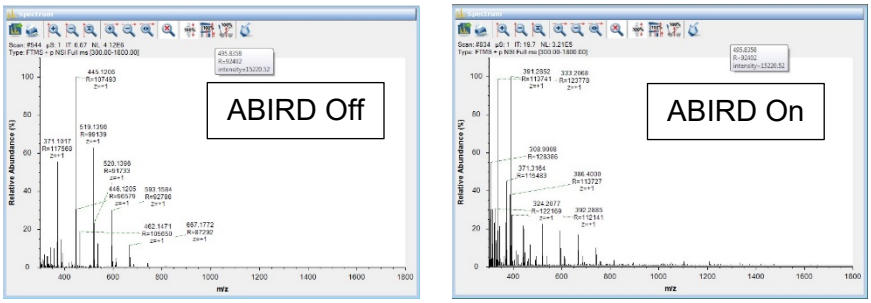
This document outlines some of these factors and how you can further optimize them to enhance peptide detection with ABIRD in use. However we can in no way guarantee this effect will be reproducible in your laboratory as there are simply far too many reasons the entire LC-MS system could be operating sub-optimally and therefore not be able to demonstrate improved peptide detection with ABIRD in use.

First important point is a correct installation. This is usually a simple task of installing the device to position the outlet tube close to the instrument inlet. The Teflon tubing is resistant to the inlet-temperatures, and it is fine to position it close or even touch the heated inlet surface. This will fully flood the inlet with cleaned air and exclude most background ion signals. The installation can be done in a variety of ways, and custom source installations are rarely a problem with flexible installation possibilities.



Next the position of the spray tip needs to be optimized, as the gentle but consistent outflow of the ABIRD needs to be compensated for. This can best be done by infusing a tune solution via the nanospray tip at normal nanospray flow rates and spray voltages while tip position is adjusted. Generally a low percentage of the spray plume is sampled, and inserting the tip too close to the inlet reduces drying time of the droplets and thus reduces signal.

With ABIRD installed, observing the reduction in background ion signal is as simple as allowing the LC to reach initial equilibration (low % organic) then turning ABIRD on and off as you observe the signal in full scan mode across a wide mass range. Take note of the background ion noise level without ABIRD on, this is the approximate signal intensity level you will need to reach during these experiments. Introducing a very intense signal well above this level will not show more identified peptides by the use of ABIRD.



After this has been done, you can begin to design your experiments to optimize data acquisition, LC conditions, instrument settings and data analysis parameters to demonstrate improved peptide detection system-wide. Some of the parameters you must investigate include, but are not limited to:

1. Instrument method parameters must be optimal. Minimum target values, maximum inject times, Top 20 vs Top 30 settings, dynamic exclusion settings, collision energies, fragmentation modes, excluded charge states all need to be examined. A high sensitivity method will have several changes from a normal ID method, each instrument specific.

2. If you are using the same method with Lock Mass (esp. Orbitrap) on, then you are wasting duty cycle looking for your low level lock mass when ABIRD is on, so make a method that saves that duty cycle can help find more peptides.

3. If you are DB searching only strict enzyme settings, you would not identify the vast number of partial cleavages that really exist at low levels.

4. If your spray tip is not spraying consistently across the gradient, you will not find the low level peptides he is looking for. Stable consistent spray is mandatory for high sensitivity analysis.

5. Chromatography is KEY! If you are running a very complex mixture on a short gradient with an overloaded column you will certainly miss the low level peptides. We have found that most labs have a vast amount of improvement they can do on their chromatography systems and it can often make a significant difference in the overall laboratory operations.

6. Your nanoLC MS system may leave room for improvement (tune and cal) or old multipliers / MCP, then you will not gain much. There are about numerous parameters that could be off on the MS instrument itself preventing that you see a benefit on Protein IDs.

Running daily standards for chromatography and peptide sensitivity to evaluate your instrument is mandatory We have standard mixes available and are happy to supply them so we can do a real sensitivity check on your instrument as it stands and after optimizations. Contact us for details.

Think about it like this. ***You need to present to the instrument more unique features / scan than it can isolate and analyze with the background ions present.*** When the extra ions are removed, the instrument has extra duty cycle to find the lower level peptides you are looking for. Also, without the high level background ions, it will fill to normal capacity with whatever is left, and actually see peptides it did not previously. This is a balance between how bad the background ions are and how well your instrument is optimized for the reduced background situation. All this is 100% user dependent / instrument dependent / problem dependent / data analysis dependent and obviously ABIRD simply cannot control for all this.

The newer & faster the instrument is, the more difficult it will be to demonstrate it.

An ABIRD on an old LTQ or LTQ-Orbitrap will make a much more significant impact than the new fast instruments.

Keep in mind that this is an effect only observed in very low level complex mixtures of peptides. Peptide signals that approach background ion level signals will be most improved.

Please review this document and provide feedback and comments on it as you see fit. We are happy to discuss these topics at length, and review of your raw data file may be requested to help this discussion.

We look forward to your feedback and hope to make a difference in your daily lab operations.