



Guide to Troubleshooting Low ETD Reagent Ion Signal

Tips for ETD Troubleshooting

- Instrument Error: Filament Fault
 - Replace filament
 - If filament fault occurs more than once every 3 months with regular use, see slide 4
- Reagent ion (m/z 202) intensity is lower than expected
 - Note: at least $5e5$ in profile mode is expected for acceptable injection time for LC-MS experiments
 - Refer to Steps to Improve Low Reagent Ion Signal on slide 3
- Signal intensity of reagent ion (m/z 202) drops significantly within 30 minutes
 - Reagent vial empty, replace with a new reagent vial (see additional slide set)
- Instrument Error: ETD Ion Gauge pressure too low
 - Check for blocked reagent needle, restrictor, or transfer line

Steps to Improve Low Reagent Ion Signal: m/z 202 signal below $5e5$ or max injection time is reached

- 1. If you have a tune file previously used for ETD with good reagent ion signal, load this tune file**
 - As ETD tune settings are saved to an individual tune file, sometimes this alone will be sufficient to improve the signal
 - Ensure the filament has been on for approximately 30 minutes
- 2. Tune ion optics**
 - ~10 min
 - Note: If you turn HCD gas off, you will need to re-tune the ion optics for the ETD reagent ions
- 3. Optimize CI gas pressure**
 - ~10 min
 - It might be necessary to retune the ion optics after the CI gas is optimized. If so, repeat step 2 until improvement in signal is less than 20%

If signal is still below $5e5$ – continue on to steps 4-6

- 4. Replace or clean the ion volume**
 - Once finished, repeat steps 2-3
- 5. Replace the ETD filament**
 - Some filaments bend, which causes low signal
 - Repeat steps 2-3
- 6. Replace reagent vial**
 - This should always be the last thing to replace. The vial should last for at least 6 months
 - Remember, do not put the used vial back even if there is still reagent left. Always insert a new vial

Filament Needs Replacing Too Often

- Filaments need to be replaced approximately once every three months with steady use
- If the filament regularly needs replacing more often
 - Check for possible leaks
 - Indications of a leak are a reagent ion gauge pressure ($>40 \times 10^{-5}$ Torr) or high required CI gas pressure (>25 psig)
 - Carrier (CI) Gas – Ensure you are using only ultra pure (99.999% pure) Nitrogen/Helium gas tank
 - Check for leaky vial septa
 - Septum should be punctured only once otherwise it may leak
 - Never remove and then reinsert the same fluoranthene vial
 - Service engineer can check for leaks in the ETD system lines if this is the suspected issue
- Past filament reliability issues have been resolved
 - Previous rhenium wire was bending
 - New filaments are not suffering from this issue

Tips for Troubleshooting

- Restrictor Temp & Transfer Line Temp heaters do not switch on
 - Reset instrument
- 202 signal disappeared, but filament is fine
 - Reset instrument
- Check target values and max. IT times after source (HESI, NSI, etc..) changes
- High reagent ion gauge pressure(>40)
 - Indication of leak
- High CI gas pressure required
 - Indication of leak

Summary

- Reagent ion intensity is important for good ETD performance. Tuning and optimization of ETD source is necessary when 202 signal is lower than expected.
- There are flexible software features for different types of ETD related experiments.
- Proteome Discoverer software and ProSightPC software are powerful tools for ETD data analysis.