

Product Information

IROA TruQuant IQQ Workflow Kit

Supplied by IROA Technologies, LLC.

Catalog Number **WORKFLOW**

Storage Temperature $-70\text{ }^{\circ}\text{C}$

Product Description

The IROA TruQuant IQQ Workflow Kit is a high-quality quantitation system for making simultaneous accurate biological measurements on several hundred biochemicals in small quantities of biological samples.

TruQuant IQQ employs isotopic peak patterns to remove artifacts and noise, eliminating false discovery. The kit provides a complex internal standard (600+ compounds) for suppression-correction, and provides suppression-corrected values and normalized data.

WORKFLOW comes with ClusterFinder™, a software tool that builds libraries, identifies/quantitates compounds and normalizes the data generated using WORKFLOW. For further information on the software, to download, and for manual and video links please visit:
<http://www.sigmaaldrich.com/catalog/product/sigma/workflow>

Components

WORKFLOW contains the materials and tools for the analysis of 90 experimental samples. The kit is intended to be used for mass spectrometry metabolomics applications.

WORKFLOW includes:

- 3 vials of lyophilized Internal Standard (IS); U-¹³C, 95% labeled (WORKFLOW-A)
- 3 vials of lyophilized Long-Term Reference Standard (LTRS); paired U-¹³C, 95% and 5% labeled; mixed 1:1 (WORKFLOW-B)
- ClusterFinder software (including library of peaks in the Long-Term Reference Standard and their physical characteristics)

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Long-Term Reference Standard (LTRS):

Aliquots of the Long-Term Reference Standard are analyzed together with experimental samples spiked with the Internal Standard.

It is used to build a “dictionary” of RT, *m/z*, and physical characteristics including fragmentation data.

The LTRS may be used as a QC standard to account for fluctuations in chromatography, mass or retention time drift, and source or instrumentation malfunctions.

The Long-Term Reference Standard is always the same and the catalog of all peaks found in each daily analysis provides a way to quantitate the performance characteristics for the instrumentation for everyday analysis and a mechanism for correcting any instrumental error.

It is used for identifying all of the compounds. Relative quantitation is sufficient, fewer scans are needed to establish quantitative accuracy, and these scans may be used to support compound identity verification. Therefore, the Long-Term Reference Standard may be used with alternating scans devoted to the collection of secondary compound identification information, such as fragmentation, ion mobility, etc., while in the analysis of the clinical/experimental samples the alternating scans may be used to get better peak shape and, thus, better quantitation.

1. Add 40 μL of injection solvent to the vial containing the LTRS. Pipette up and down to ensure solubilization of the LTRS material. Keep on ice until use.
2. Use 3 μL for LC-MS analysis. Note: Some Users prefer to add 2 μL for positive mode and 4 μL in negative mode.

Internal Standard (IS):

The Internal Standard is the 95% U-¹³C labeled component of the Long-Term Reference Standard only and is used as a yardstick. It provides enough information for complete identification and quantitation of samples without the need for chromatographic baseline correction. Since the chemical makeup and chromatographic behavior of the Long-Term Reference Standard sample is identical to the Internal Standard, it is possible to use the in-depth and informationally strong, triply redundant chemical information obtained from the Long-Term Reference Standard sample collected in the dictionary and apply it to identify compounds in the experimental sample.

It may be used to align all the peaks in the chromatogram and used as an injection standard.

The Internal Standard is incorporated into the re-solvation solvent. The material in each amber vial is expected to be suitable for 30 samples.

1. Re-solvation solvent is either the initial chromatographic solvent or ultrapure water. (Where the chromatographic solvent contains acidic ultrapure water is preferable to reduce degradation prior to injection, but either will work).
2. Add 1.2 mL of re-solvation solvent to the amber vial, mix, and then vortex. This is the Internal Standard solution.
3. Hold the Internal Standard on ice until ready to re-solvate the samples.
4. To each dried sample add 40 µL of the Internal Standard, then vortex.
5. If you have a filtration step, then filter before analysis after re-solvation.

Note: A “pre-prep” normalization should be performed on each sample prior to crash. The recommendation is that a protein reading be done on each sample homogenate, and equal amounts of protein be delivered as the “prep sample”. While this may not always be appropriate it is always better to collect all the information one can on every sample and attempt a prep-normalization. The above protocol delivers ~2 µg each of U-95% ¹³C labeled and U-5% ¹³C labeled (Long-Term Reference Standard) and 2 µL U-95% ¹³C labeled (Internal Standard) per sample which should be appropriate for most MS platforms; however, it is recommended that a “calibration” step is done ahead of time, i.e. test different amounts of standard prep with the same amount of Internal Standard to figure out how much to balance with the Internal Standard. Please see Appendix, IS Calibration section.

Storage/Stability

Store the kit at –70 °C.

Procedure

Build Dictionary using the Long-Term Reference Standard data files. Identify compounds, fragments, and adducts using ClusterFinder:

1. Upload the Long-Term Reference Standard LC-MS data files to IROA ClusterFinder. ClusterFinder uses algorithms to build, evaluate, edit, and export the result of an untargeted search. Refer to ClusterFinder user manual and instructional videos, provided separately.
2. Connect the relevant Long-Term Reference Standard library, likely Long-Term Reference Standard-pos, or Long-Term Reference Standard-neg. These libraries are devoid of retention times but are a good starting point. If available, attach method specific libraries (see last step) instead as it will contain previously recorded retention times.
3. Compounds, fragments, and adducts are identified in minutes using algorithms that search for the specific signatures.
4. Curate the data. Examine spectrum bins and their constituent IROA peaks. Look at the detected features in the context of underlying raw data and manually correct the results in cases where the automatic algorithm has failed. The curation process takes approximately an hour to complete.
5. Build a targeted library (.cflib file) from each method or LC-MS mode. Each library will be used to perform a targeted search of the clinical/ experimental Internal Standard-containing files run coincidentally to the Long-Term Reference Standard.
6. Create a method specific dictionary as a ClusterFinder database:
 - a. Disconnect the Long-Term Reference Standard dictionary from the search.
 - b. Create a new library (database) and attach it to the method of analysis.
 - c. Select all bins in the curated method.
 - d. Right-click the selected bins, and select “add to Library”.
 - e. The new library will now have retention times, spectra, and all related information, and is likely the dictionary to connect in future work.
7. Export the resultant dictionary in comma separated value (CSV) format or compound exchange format (CEF) for storage or transport to other ClusterFinder installations.

Identify compounds, fragments, and adducts in the experimental samples using ClusterFinder and the Long-Term Reference Standard dictionary:

1. Upload LC-MS data files to ClusterFinder.
2. Run a targeted analysis using the targeted library (.cflib file) that was created following the curation of the Long-Term Reference Standard files.
3. Compounds, fragments, and adducts are identified in minutes using algorithms that search for the specific signatures.
4. Curate the data and examine spectrum bins and their constituent peaks. Look at the detected features in the context of underlying raw data and manually correct the results in cases where the automatic algorithm has failed. The curation process takes approximately an hour to complete.
5. Export the results as tab separated value (tsv) files.
6. The quantitative output of targeted analysis, together with experiment design may serve as input for the statistical and biochemical interpretation.

Appendix

Internal Standard (IS) Calibration

The calibration of IS to your SOP simply requires the generation of a single large portion of prepared "prepped" sample (for example, plasma, cells or tissue), as many as 20× the normal sample size is usually sufficient.

1. Pool and filter prepped sample to create a single homogeneous sample.
2. Deliver differing aliquots of the pooled sample, ranging from half of the normal amount to 4 times the amount normally delivered to capped sample tubes. Make triple samples.
3. Dry aliquots under a gentle nitrogen stream.

4. Add 1.2 mL of ultrapure water to a single vial of IS. Thoroughly mix to ensure a single homogeneous solution. (Note: It should go into solution freely). Keep on ice until ready to use.
5. Use a 40 µL aliquot of the IS to resolute each of the dried samples. Upon addition of IS, mix thoroughly.
6. Resolute a single vial of the Long-Term-Reference Standard (Matrix) with 40 µL of ultrapure water, thoroughly mix to ensure complete solvation. Keep on ice until ready to use.
7. Analyze samples using the chromatographic method you would normally use. The analysis may be repeated in multiple modes, i.e. positive reverse phase, negative reverse phase, positive HILIC, or negative HILIC.
8. Use the ClusterFinder software to find and identify all peaks in the Long-Term Reference Standard in an unbiased analysis.
9. Export the compounds found in the previous step to run a targeted analysis of the IS containing samples. Use the ClusterFinder software to export all of the quantitative data from the IS containing samples for statistical analysis. Export all of the compound identification and QA/QC data from the Long-Term Reference Standard.
10. Analyze the samples to identify the amount of the prepped samples that yields an overall mass spectral signal that is equal to the overall mass spectral signal of the IS. This is the amount of sample that will most accurately be measured using the IS in the future, i.e. well balanced by the standard 40 µL of IS.

ClusterFinder is a trademark of IROA Technologies, LLC.

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