



Mass Spectrometry / Frequently Asked Questions and Answers

Q: How do I know if APAF has received my sample for MS services?

A: Upon receiving samples, APAF will log the service request and set a project code for the service requested. An e-mail will be automatically generated and sent to you notifying you that your sample for the project has been received and has been entered into the analysis queue. The email contains a project code that you should refer to during subsequent enquires. The e-mail is also sent to the APAF staff that will be responsible for the project. Customers should not reply directly to this email as they can not be viewed by our service staff. Please contact our staff directly or call 02 9850 6201 for sample enquires.

Q: When do I expect to have the result after submitting sample?

A: All samples are placed into a queue for analysis based on sample receipt date. Please [contact us](#) prior to sending your sample if you need your sample to be serviced urgently.

For MALDI MS and 1D nanoLC ESI MS/MS analysis for protein identification, we aim to have the report sent to you in electronic format within 15 working days after receiving the sample.

For intact protein mass analysis, the report will be sent to you within 10 working days.

Non routine services generally take longer times.

Please be aware that mass spectrometers can occasionally experience downtime that is beyond the control of APAF staff. Delays can occur as replacement parts must be imported from overseas and fitted by MS service vendors. Depending on the type of MS service requested, some customers may experience delays in sample processing during these instrument downtimes. In the case of any extensive delay APAF staff will notify the customer and advise them of the length of such delay.

Q: How do I know what service to select?

A: Please visit the [MS Services](#) section in this web site for detailed explanations on the mass spectrometry services offered by APAF. Please contact us if you require further information.

Q: How much sample is needed for the service?

A: This depends on the type of services.

For intact protein mass analysis using ESI MS technique, 1 mg of solid sample is normally sufficient. For liquid samples, we recommend the protein concentration to be no less than 5 pmol/ul and no less than 20 ul should be send to us.

For 2D LC ESI MS/MS of complex protein samples extracted from cell lysate, tissues, body fluids, or other biological sources, 50 ug up to 200 ug of total proteins would be needed.

For 1D LC ESI MSMS of complex protein samples, 2 ug up to 50 ug of total proteins would be needed.

For protein identification from 2D gel spots, we recommend you cut and submit the whole spot for medium and faint stained spots. Provided that you can accurately detect the spot from the gel, we can usually provide useful spectral data. Please be aware that keratin contamination is a commonly problem seen with faint gel spots (i.e. ratio of keratin to protein of interest is high). Take precautions when excising gel spot. Keratin contamination can be introduced from skin flakes, hair, dusty lab environments, contaminated excision instruments, etc.

Q: How do I send samples to APAF?

A: You can post your sample to us in a safe mailing envelop with the completed MS Service [Request Form](#) and purchase order form. You may also send samples with the forms by courier.

Dried or gel samples can be shipped at room temperature.

Liquid samples should be shipped frozen in dry ice.

If you are sending samples from overseas, it is essential that you visit the [Quarantine Guideline](#) section on this web site and accurately follow the quarantine instructions when sending samples.

Frequently asked questions (FAQs) related to iTRAQ

Q. What is iTRAQ?

A. iTRAQ is a chemical labelling multiplexing technique that uses mass spectrometry for relative protein quantitation. iTRAQ is commercialised by Applied Biosystems Inc.

Q. How many samples can be analysed by iTRAQ?

A. iTRAQ is currently available as a 4-plex kit. Because it is a relative quantitation technique, 1 of the 4 channels is used as a reference sample to provide relative quantitation between the other 3 channels.

ABI has announced plan to introduce an 8-plex kit in late 2006.

Q. What should be used as the reference sample?

A. Some suggestions for the reference sample are (i) a pooled sample made by combining an aliquot of individual samples that will be monitored in the other channels, (ii) an individual sample used as the reference sample.

As the reference sample is required for each iTRAQ experiment, it is important that sufficient sample is available to complete a large iTRAQ experiment.

Q. How much sample is required?

A. 5ug to 100ug proteins can be labelled with each iTRAQ reagent. 50uL of plasma is required for immunodepletion and 1 iTRAQ labelling. 1mL CSF is required for immunodepletion and 1 iTRAQ labelling.

Q. Is immunodepletion necessary when using iTRAQ with biofluids such as plasma?

A. Absolutely. Otherwise the number of identified proteins is small.

Q. How should the sample be prepared for iTRAQ labelling?

A. We accept biological samples such as cell lysate, tissues or body fluids. We also accept the extracted proteins in solid or liquid. When sending us extracted proteins, please make sure no primary amines are added in the samples and the salt concentration is reduced as much as possible. Samples and the controls need to be prepared in the same way and the total protein quantities in the different labels need to be in the similar level. If you are to prepare iTRAQ labelling yourself, you should follow the instructions of the iTRAQ reagent kit and send us dried samples which has been labelled but not been cleaned up using strong cation exchange chromatograph.

Q. What is APAF's standard workflow for iTRAQ?

A. The protein sample is solubilized, reduced and alkylated then digested with trypsin. The tryptic peptides are labelled with iTRAQ reagent and mixed at a ratio so that the protein quantities in the different labelled samples are similar. The mixed sample is cleaned or cleaned/fractionated by SCX. Each fraction is separated by reversed-phase gradient and injected into a Q-Star XL mass spectrometer. The data is analysed for protein identification and relative quantitation by ProteinPilot software (ABI) and a report is provided to the client.