

Frequently asked questions (FAQs) iTRAQ™,

Q. What is iTRAQ™?

A. iTRAQ™ is a chemical labelling multiplexing technique that uses mass spectrometry for relative protein quantitation. The iTRAQ™ reagent 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. If more than four samples are to be analysed, a common reference sample should be used in all subsequent experiments.

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 for all iTRAQ™ runs.

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. 5 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 bio-fluids such as plasma?

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

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

A. All samples should be treated in exactly the same manner to minimise quantitation errors introduced during protein extraction and purification. Primary amines such as tris and ammonium bicarbonate must be avoided in sample preparation as it will interfere with the iTRAQ™ labelling. APAF prefers that customers send unlabelled samples so that iTRAQ™ labelling can be conducted on-site at APAF. For protein solubilisation customers should use 0.5M triethylammonium bicarbonate. 0.1% SDS can be used to aid protein solubility. If liquid protein samples are to be sent for iTRAQ™ analysis, the sample concentration should be ~ 5mg/mL and the volume <100uL. Plasma samples should be sent directly with no prior manipulation.

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

A. The protein sample is solubilised, reduced and alkylated then digested with trypsin. The tryptic peptides are labelled with iTRAQ™ reagent and a small aliquot of each sample is mixed at a ratio of 1:1:1:1. This sample is analysed by LC-MS/MS to check the mixing ratios are accurate. Based on this result the remaining samples are mixed in appropriate ratios to ensure equal loading. The mixed sample is separated into 15-20 fractions by SCX. Each fraction is separated by 2h reversed-phase gradient and MSMS data acquired on Q-Star XL mass spectrometer. The data is analysed for protein identification and relative quantitation by ProteinPilot™ software (ABI) and a report provided to the client.