Expression Pathology

Microproteomic Profiling From Formalin Fixed Tissue

iTRAQ™ Labeling of Liquid Tissue® MS Protein Preparations

Introduction

iTRAQ® reagents (Applied Biosystems) are multiplexed stable isotope reagents that can label all peptides in up to four biological samples enabling peptide identification and relative quantitation. The samples are labeled individually and then mixed and analyzed simultaneously in one LC-MS/MS run.

The Liquid Tissue MS Protein Prep Kit can be used with iTRAQ labeling reagents, with the following modifications to the standard Liquid Tissue protocol:

1. For a standard Liquid Tissue reaction, approximately 30,000 cells are procured from a tissue section and suspended in 20 µl Liquid Tissue Buffer.
2. Heat the preparation at 95°C for 90 minutes.
3. After cooling for 2 minutes on ice, add 1 µl of Trypsin Reagent and vortex.
4. Incubate at 37°C for one hour with vigorous shaking for one minute at 20 minute intervals.
5. Incubate at 37°C for 16 to 18 hours.
6. Microcentrifuge the tube(s), 10,000 rcf for 1 minute.
7. Heat the Reaction Tube(s) at 95°C for 5 minutes
8. Microcentrifuge the tube(s), 10,000 rcf for 1 minute.

Note: *The Liquid Tissue MS Protein Prep Kit Reduction Reagent, DTT, is not compatible with the iTRAQ labeling reaction, or with the Micro BCA Protein Assay for total protein determination. It is not used at this point in the processing of the sample.*

9. An aliquot of the reaction can be taken for determination of protein concentration by the Micro BCA Protein Assay Protocol for Liquid Tissue MS Protein Preps (see the instruction manual for the kit). Determination of protein concentration is helpful for normalizing the amount of the sample that is used in the labeling reaction. The iTRAQ labeling kit protocol uses 5 to 100 µg of sample in the labeling reaction.

Labeling with iTRAQ Reagents

1. Dry the Liquid Tissue preparation completely using a Speed-Vac.
2. Re-suspend and vortex the sample in 100 µl of Solution A (0.5% Trifluoroacetic Acid (TFA) in 5% Acetonitrile (ACN)).
3. Load re-suspended sample onto an activated PepClean™ C-18 Spin Columns (Pierce Biotechnology, Rockford, IL).
4. Wash with Solution A.
5. Elute peptides with Solution B (70% ACN)
6. Dry using a Speed-Vac.
7. Dissolve the iTRAQ reagents in ethanol.
8. Dissolve the dried Liquid Tissue sample in 30 µl of iTRAQ Dissolution Buffer.
9. Add 1 µl of iTRAQ Denaturing Reagent and vortex.
10. Add 2 µl of iTRAQ Reducing Reagent and vortex.
11. Incubate at 60°C for one hour.
12. Add 1 µl of iTRAQ Cysteine Blocking Reagent and vortex.
13. Incubate at room temperature for 10 minutes.
14. Add 70 µl of iTRAQ reagent to the sample and vortex.
15. Incubate at room temperature for 1 hour.
16. Add 200 µl of HPLC grade water to quench the reaction.
17. Incubate at room temperature for 30 minutes.
18. Vortex and store at –80°C until further processing.

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Rev. 1, April 4, 2007: iTRAQ labeling of Liquid Tissue Preps