Solution-stabilized TMT & TMTpro reagents in 96-well plates for high-throughput sample processing

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ABSTRACT
Thermo Scientific™ TMT™ and TMTpro reagents enable quantitative proteomic analysis of up to 16 samples in parallel by mass spectrometry. To facilitate high-throughput applications, we have developed a ready-to-use 96-well microplate format of solution-stabilized reagents for large-scale sample labeling using automated liquid handling platforms. We also show that these stabilized reagents in solution retain high reactivity during storage at elevated temperatures and have equivalent performance to existing dry-format samples.

INTRODUCTION
Thermo Scientific™ Tandem Mass Tag™ (TMT™) reagents enable researchers to unambiguously identify and quantify proteins and peptides from multiple samples in a single LC-MS/MS analysis. This powerful multiplexing technology has driven quantitative proteomic experiments to larger sample sizes. High-throughput processing of hundreds to thousands of samples is often facilitated by microplates and automated liquid handling platforms. For labeling multiple samples, TMT and TMTpro reagents provide dry bulk powder in tubes that are currently the preferred choice, but they must be reconstituted into microplate wells after reconstitution in anhydrous organic solvents. When done manually, this is time-consuming and susceptible to error. In addition, TMT reagents dissolved in neat organic solvent still degrade by hydrolysis from trace amounts of water within weeks or just days depending on the concentration, relative humidity, and storage temperature. Lastly, current protocols recommend acetonitrile (ACN) for reagent reconstitution, but this reagent’s volatility and low viscosity make it difficult for robotic pipettors to aspirate and dispense accurately due to dripping and evaporation.

To improve TMT and TMTpro reagent stability and handling in a liquid format, we have developed a non-volatile, moderately viscous dithiothreitol (DMDSO)-based solution with a stabilizing agent. The solution-stabilized reagents are contained into ready-to-use 96-well PCRs that are ideal for automated liquid handling. TMT and TMTpro reagents are provided as 5 µL DMDSO-based stabilization solution of 40 µg and 50 µg per well, respectively, which is suitable for labeling protein digest samples in the range of 5 µg to 70 µg. Each 96-well PCR microtiter plate contains multiple sets of reagents: 8 x TMT10plex, 4 x TMT16plex, 6 x TMTpro10plex, and 4 x TMTpro16plex (Figure 1). Plates are sealed with foil that may be pierced or opened.

MATERIALS AND METHODS
TMT and TMTpro 96-well plate preparation
TMT and TMTpro reagents were formulated at 8 and 10 µg/µL, respectively, in DMDSO-based stabilization solution and dispensed into 96-well PCR microwells at 5 µL per well. Plates were then reconstituted with foil and stored in foil pouches prior to use.

Sample Preparation
Human HeLa S3 cells were grown in αMEM supplemented with 10% FBS, 1x Glutamine and 1% Pen/Strep. HeLa digest samples were prepared using the Thermo Scientific EasyPep™ MS sample prep kit protocol. Peptides were reconstituted in 100 mM TEAB buffer pH 8.5, labeled for 1 hr with TMT and TMTpro reagents in DMDSO-based stabilization solution (or ACN for control), and cleaned up using the EasyPep SPE protocol.

LC-MS/MS analysis
Samples were analyzed by LC-MS/MS using a Thermo Scientific™ Orbitrap Eclipse™ Tribrid™ mass spectrometer interfaced with a Thermo Scientific™ Donnies™ 3000 RSL-Orbitrap system using a 50mC18 Thermo Scientific™ EasySpray™ column with a gradient elution of 3:25% ACN with 0.1% formic acid over 120 min at a flow rate of 300 nl/min. FT-MS spectra (RP 1200) and HCD FT-MS spectra (RP 15K) were acquired as 3x3 DDA scan cycle with 50% dynamic exclusion.

RESULTS

Figure 1. Solution-stabilized TMT and TMTpro reagents in 96-well PCR plates

Figure 2. TMTpro labeling – ACN vs. DMDSO-based stabilization solution

Figure 3. TMT labeling – HeLa digest sample amount range

Figure 4. TMTpro labeling – HeLa digest sample volume range

Figure 5. Reagent stability in DMDSO-based stabilization solution – storage

CONCLUSIONS
TMT and TMTpro reagents in DMDSO-based stabilization solution retain high reactivity during storage at elevated temperatures, freeze-dry, and use in open air on bench.

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PC2203-621N

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