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

Dustin Frost, Amarjeet Flora, Leigh Foster, Ryan Bomgarden Thermo Fisher Scientific, Rockford, IL, USA

ABSTRACT

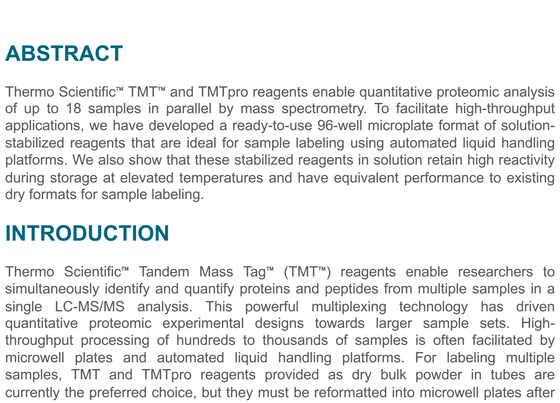
Thermo Scientific™ TMT™ and TMTpro reagents enable quantitative proteomic analysis of up to 18 samples in parallel by mass spectrometry. To facilitate high-throughput applications, we have developed a ready-to-use 96-well microplate format of solution-stabilized reagents that are ideal for sample labeling 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 formats for sample labeling.

INTRODUCTION

Thermo Scientific™ Tandem Mass Tag™ (TMT™) reagents enable researchers to simultaneously identify and quantify proteins and peptides from multiple samples in a single LC-MS/MS analysis. This powerful multiplexing technology has driven quantitative proteomic experimental designs towards larger sample sets. High-throughput processing of hundreds to thousands of samples is often facilitated by microwell plates and automated liquid handling platforms. For labeling multiple samples, TMT and TMTpro reagents provided as dry bulk powder in tubes are currently the preferred choice, but they must be reformatted into microwell plates after reconstitution in anhydrous organic solvent. 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 tag concentration, relative humidity, and storage temperature. Lastly, current protocols recommend acetonitrile (ACN) for reagent reconstitution, but this solvent'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, moderate viscosity dimethyl sulfoxide (DMSO)-based solution with a stabilizing agent. The solution-stabilized reagents are configured into ready-to-use 96-well PCR microplates that are ideal for automated liquid handling. TMT and TMTpro reagents are provided in 5 µL DMSO-based stabilization solution at 40 µg and 50 µg per well, respectively, which is suitable for labeling protein digest samples in the range of 1 µg to 10 µg. Each 96-well PCR microplate contains multiple sets of reagents: 8x TMT 10plex, 8x TMT 11plex, 6x TMTpro 16plex, and 4x TMTpro 18plex (Figure 1). Plates are sealed with foil that may be peeled or pierced.

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



MATERIALS AND METHODS

TMT and TMTpro 96-well plate preparation

TMT and TMTpro reagents were formulated at 8 and 10 µg/µL, respectively, in DMSO-based stabilization solution and dispensed into 96-well PCR microplates at 5 µL per well. Plates were heat-sealed with foil and stored in foil pouches prior to use.

Sample Preparation

HeLa S3 cells were grown in sMEM supplemented with 10% FBS, 1x Glutamax 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 DMSO-based stabilization solution (or ACN for control), and cleaned up using the EasyPep SPE protocol.

LC-MS/MS analysis

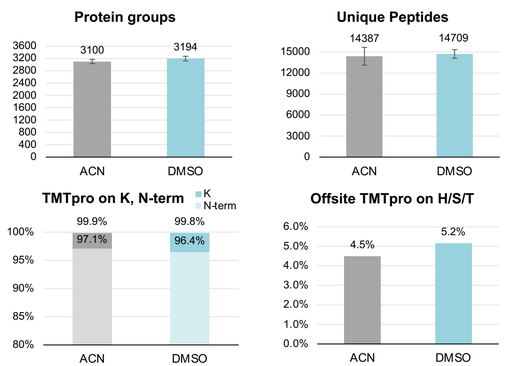
Samples were analyzed by LC-MS/MS using a Thermo Scientific™ Orbitrap Eclipse™ Tribrid™ mass spectrometer interfaced with a Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLCnano system using a 50cm C18 Thermo Scientific™ EASY-Spray™ column with a gradient elution of 2-32% ACN with 0.1% formic acid over 120 min at a flow rate of 300 nL/min. FT-MS scans (RP: 120K) and HCD FT-MS^2 scans (RP: 15K) were acquired using a 3 sec DDA scan cycle with 60 sec dynamic exclusion.

Data Anaysis

Raw data was processed by Thermo Scientific™ Proteome Discoverer™ 3.0 using the SEQUEST® HT search algorithm with an UniProt human protein database. Peptide modifications consisted of static carbamidomethylation (C), dynamic oxidation (M), dynamic deamidation (N, Q), and either static or dynamic TMT or TMTpro tags (N-terminus, K). Protein and peptide identifications were filtered to a 1% FDR threshold using Percolator.

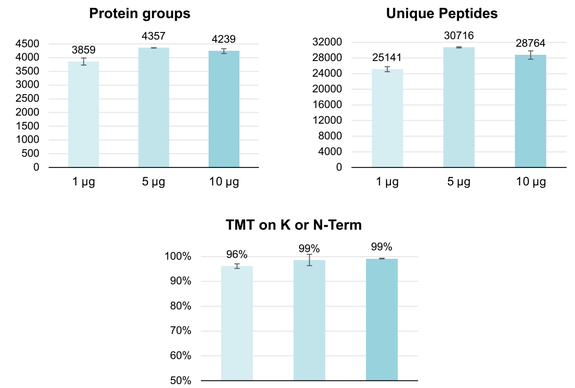
RESULTS

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



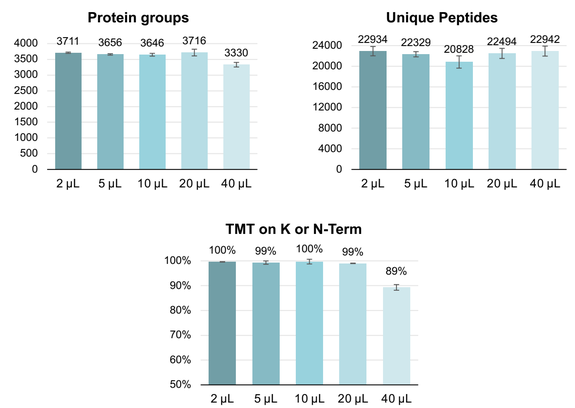
HeLa digest (5 µg) in 100 mM TEAB buffer pH 8.5 was labeled with TMTpro reagents in either ACN or DMSO-based stabilization solution for 1 hr at 8:1 tag:peptide ratio (w/w) in 96-well PCR plates (in triplicate; channels 126, 130N, 134N), cleaned up using EasyPep SPE resin, and samples were acquired by LC-MS/MS (500 ng injection). Numbers of identified protein groups & unique peptides, labeling efficiency on lysine residues (K) and N-termini, and offsite labeling are equivalent between samples labeled by TMTpro reagents in either ACN or the DMSO-based stabilization solution.

Figure 3. TMT labeling – HeLa digest sample amount range



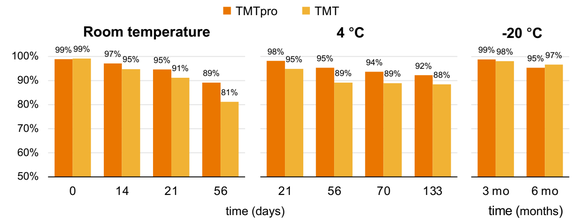
HeLa digest samples (1, 5, or 10 µg) were prepared using the EasyPep 96 micro kit protocol and labeled in triplicate with TMT zero (40 µg) in 5 µL DMSO-based stabilization solution for 1 hr. LC-MS/MS analysis was performed to identify protein groups & unique peptides and determine overall labeling efficiency (1 µg sample = 400 ng per injection; 5 and 10 µg samples = 1 µg per injection).

Figure 4. TMT labeling – HeLa digest volume range



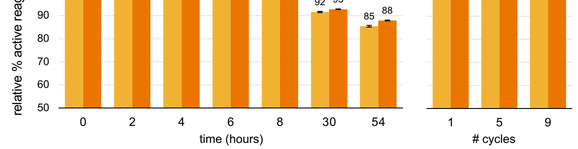
HeLa digest samples (1 µg) in 2, 5, 10, 20, 40 µL 100 mM TEAB buffer pH 8.5 were labeled in triplicate with TMT zero (40 µg) in 5 µL DMSO-based stabilization solution for 1 hr and cleaned up using the EasyPep 96-well peptide clean-up plate. LC-MS/MS analysis was performed to identify protein groups & unique peptides and determine overall labeling efficiency (400 ng per injection).

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



TMT (40 µg) and TMTpro (50µg) reagents in 5 µL DMSO-based stabilization solution in foil-sealed 96-well PCR plates were incubated at room temperature, 4 °C, and -20 °C to evaluate stability over time. At various time points, wells were punctured, reagents were diluted 1:500 v/v in ACN:H_2O, and direct infusion MS spectra were acquired on a Q Exactive™ HF MS to measure the parent ion intensities of the active reagent (NHS) and the inactive hydrolyzed form (acid). The percent active reagent was calculated by (NHS)/(NHS+acid) × 100%. Reagents remain stable over many weeks, even at room temperature.

Figure 6. Reagent stability in DMSO-based stabilization solution – in-use



TMT (40 µg) and TMTpro (50µg) reagents in 5 µL DMSO-based stabilization solution in unsealed 96-well PCR plates were incubated at room temperature to evaluate in-use stability. Freeze-thaw stability was also assessed in a sealed plate over 9 cycles (measurement by infusion MS as above). The percent active reagent is reported as relative to the initial amount (100%). Reagents remain stable after many freeze-thaw cycles, and exposed reagents in unsealed plates retain high reactivity for more than 8 hours.

CONCLUSIONS

- TMT and TMTpro reagents in the DMSO-based stabilization solution retain reactivity during storage at elevated temperatures, freeze-thaw, and use in open air on bench
- Reagents in the DMSO-based stabilization solution perform equivalently vs. in ACN
- Solution-stabilized reagents in the 96-well PCR microplate format efficiently label complex protein digest samples (>98%) for sample ranges of 1-10 µg

TRADEMARKS/LICENSING

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