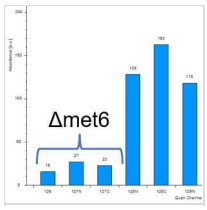

Feb 01, 2022

pasefRiQ_V1

lcmsmethods [1], Yuting Yuan[1], Namandje' N Bumpus[1]

[1]The Johns Hopkins University School of Medicine

1

dx.doi.org/10.17504/protocols.io.b4ifqubn

LCMSMethods.org Version 2

lcmsmethods

Citation: lcmsmethods , Yuting Yuan, Namandje' N Bumpus pasefRiQ_V1 https://dx.doi.org/10.17504/protocols.io.b4ifqubn

We have developed optimized methods for reporter ion quantification on a TIMSTOF Flex (TIMS B configuration) by iterative analysis of the TMT triple knock out yeast standard and an in-house developed two-proteome standard. For the greatest reduction in background coisolation interference in the TMT6 and TMT10/11 plex standards, 10 ion mobility ramps from 0.8 to 1.3 1/k0 provides the greatest reduction in interference on 60 minute LC gradients when using a 25cm IonOpticks aurora column. Reducing the number of ramps to 5 leads to an overall increase in peptide identifications with a loss in unique protein identifications. It is worth noting that the distribution of 1/k0 of peptides labeled with the TMT6/10/11plex and TMTPro reagents have somewhat different overall distibutions. For the maximum intrascan linear dynamic range. For 30 minute pasefRiQ experiments, 10 ramps at a 2.2 second cycle time has a detrimental effect on the overall identification of PSMs, peptides and proteins.
For the highest possible coverage of each protein identified with the TMTPro reagents on 30 minute gradients we recommend 5 ramps using a 1/k0 range of 0.7 to 1.4.
Typical results for pasefRiQ using a two proteome standard as described is 3,600 quantified proteins in 60 minutes with a 240ng total load on column.
At 24ng load on column, this same method with no alterations will return approximately 2,100 quantified proteins. Increasing the multiplier voltage with the high sensitivity model will result in an approximately 15% more peptides and proteins identified at this load.

pasefRiQ_methods_2_1_2022.zip

DOI

dx.doi.org/10.17504/protocols.io.b4ifqubn

lcmsmethods , Yuting Yuan, Namandje' N Bumpus 2022. pasefRiQ_V1. **protocols.io** https://dx.doi.org/10.17504/protocols.io.b4ifqubn

NIH
Grant ID: R01AG064908

NIH
Grant ID: R01GM103853

protocol ,

Feb 01, 2022

Feb 01, 2022

57639

Citation: lcmsmethods , Yuting Yuan, Namandje' N Bumpus pasefRiQ_V1 https://dx.doi.org/10.17504/protocols.io.b4ifqubn