Reference Yeast and Human Whole Cell Protein Extracts for Mass Spectrometry Instrument Performance Monitoring and Method Development

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1. Introduction

To monitor mass spec instrument performance for proteomics applications and optimize protein mass spec sample preparation, a reference protein material of high complexity is required. Whole cell protein extracts provide this desired sample complexity. However, to be compatible with mass spec applications, such extracts should meet a number of design requirements:

- ✓ *compatibility with LC/MS (free of detergents, etc.)*
- ✓ *high protein integrity (minimal level of protein degradation and non-biological PTMs)*
- ✓ *compatibility with common sample preparation methods such as proteolysis, PTM enrichment and mass-tag labeling*
- ✓ *Lot-to-lot reproducibility*

Here we describe whole cell protein extracts from yeast and human cells that meet the above criteria. Two extract formats have been developed:

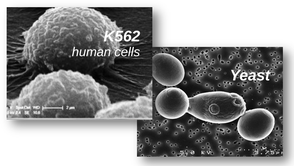
- *Pre-digested extracts (peptides); primary use - instrument performance monitoring*
- *Intact protein extracts; primary use – optimization of sample preparation*

2. The extract features

Source

Human extract - from K562 cells
Complex human proteome with a large dynamic range.

Yeast extract - from *Saccharomyces cerevisiae*
~6,600 ORFs; all proteins quantified previously.



Extract preparation

Cell lysis

- The process is optimized to allow for minimal protein fragmentation.
- Instant inactivation of endogenous proteases to assure virtual lack of nonspecific cleavages.
- *Only the reagents compatible with LC and MS are used.*

Sample preparation and digestion

- Strict control over the procedural steps assures that non-biological PTMs are maintained at lowest level.
- Proteins are exhaustively digested (less than 8% of missed tryptic cleavages).

Peptide clean-up and lyophilization

- Non-peptide material is removed with solid phase extraction (C_{18}).
- Peptides are dispensed in 100 ug aliquots with high accuracy and lyophilized for long term stability.

Mass spec analysis report for the extracts

Extract	Human	Yeast
Identified proteins	2120	1637
Total spectra	26440	21264
Spectrum match	65%	52%
Unique peptides	16375	12079
Deamidation spectra	4.9%	6.5%
Oxidation spectra	0.6%	1.3%
Carbamylated spectra	0.2%	0.6%
Nonspecific cleavages	1%	2%
Missed tryptic cleavages	4.9%	4.8%

Q Exactive (Thermo), 2h gradient
1 ug extract injection

Strict control over all the steps of preparation protocol assures that the extracts meet the critical requirements of reference materials for mass spectrometry.

3. Pre-digested extracts
Reference material for instrument performance monitoring

Features

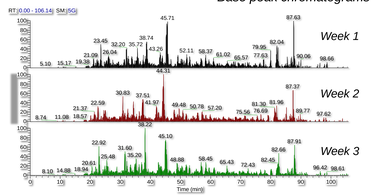
- ✓ Full compatibility with LC and MS
- ✓ Ready for analysis - *no need to reduce, alkylate, digest, or clean-up the sample*
- ✓ Efficiently digested (*less than 8% missed cleavages*)
- ✓ Highly pure *(SPE C_{18} clean-up)*
- ✓ Select material complexity you need – *relatively small yeast proteome or complex human proteome with a large dynamic range*

Applications

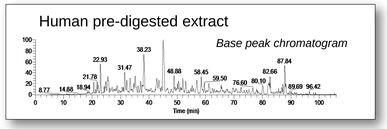
- ✓ Instrument performance monitoring
- ✓ Mass spec method optimization
- ✓ Interlaboratory instrument benchmarking

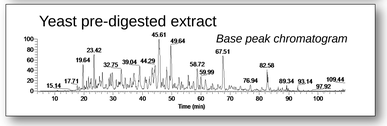
Monitoring LC/MS instrument performance (example)

Base peak chromatograms



Instrument performance (Waters NanoAquity HPLC system interfaced to a ThemoFisher Q Exactive) was monitored with human pre-digested extract (1ug injection, 2h gradient).



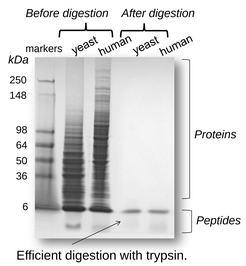


Test results by different users

Instrument, laboratory and method	Pre-digested extract	Identified unique peptides	Identified proteins
Instrument #1, Lab #1, method #1	yeast	12427	1567
	human	18040	2370
Instrument #1, Lab #2, method #2	yeast	27491	3096
	human	46018	4664
Instrument #2, Lab #3, method #3	yeast	11233	1220
	human	10344	1588
Instrument #3, Lab #4, method #4	yeast	14064	2703
	human	19531	3885

4. Intact extracts
Test material for optimization of sample preparation

Testing trypsin digestion



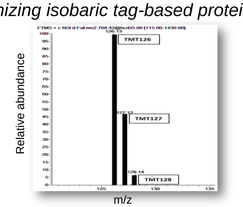
Efficient digestion with trypsin.

Validating phosphopeptide enrichment method

Extract	Total peptides	Phosphopeptides	Phosphopeptide enrichment
Yeast	2502	1715	68.5%
Human	1768	1537	87%

The extracts provided a good model system for establishing efficient phosphopeptide enrichment with TiO_2 resin.

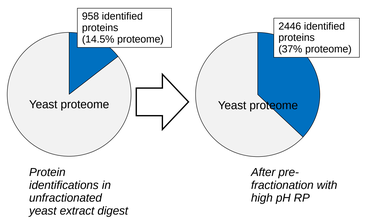
Optimizing isobaric tag-based protein quantitation



Three aliquots of the digested human extract were individually labeled with a specific TMT tag and mixed at 10:5:1 ratio.

Good correlation between observed and expected protein quantitation levels was observed.

Establishing a method for fractionation of a complex peptide mix

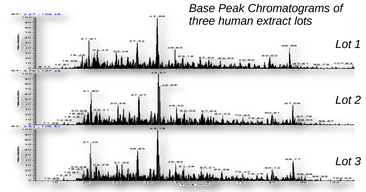


Protein identifications in unfractionated yeast extract digest

After pre-fractionation with high pH RP

4. Lot-to-lot reproducibility

Lot-to-lot reproducibility



Lot	#1	#2	#3
Identified proteins	2101	2076	2088
Unique peptides	25116	25821	25883
Deamidation spectra	4.9%	5.1%	5.0%
Oxidation spectra	0.6%	0.5%	0.6%
Nonspecific cleavages	1%	1%	1%
% missed tryptic cleavages	4.8%	4.8%	4.6%

Waters NanoAquity HPLC system interfaced to a Themo Fisher Q Exactive, 2h gradient, 1 ug extract injection.

Quantitation via SRM assay
Courtesy by Dr. Koomen, Moffitt Cancer Center

Protein	Peptide	Lot	Normalized abundance*
STAT3_HUMAN	TLTDEELADWK	**A**	**1.10**
		B	**0.83**
		C	**1.00**
STAT5A_HUMAN	LSPPAGLFTSAR	**A**	**0.92**
		B	**1.09**
		C	**1.00**
	YYTPVLAK	**A**	**1.00**
		B	**1.09**
		C	**0.93**

*GAPDH normalized data

Quantitation via spectral counting

Human extract *Spectral counting*			
protein	lots		
	#1	#2	#3
HS90B	155	157	153
ACTG	116	113	113
HS90A	134	130	129
PRKDC	126	129	116
TBB5	96	96	99
IF2P	18	17	17
ANXA2	21	23	23
DLDH	19	16	18
PDIA4	21	27	22

Yeast extract *Spectral counting*			
protein	lots		
Protein	#1	#2	#3
YBR118W	358	375	368
YGR192C	356	344	335
YCR012W	369	336	304
YBR196C	153	153	146
YLR249W	130	129	128
YDL145C	23	22	25
YDR261C-D	31	30	28
YKL035W	31	26	32
YOR375C	28	32	28

Lot-to-lot extract reproducibility with respect to protein composition and abundance

5. Conclusions

- ❑ **We developed MS ready whole cell yeast and human protein extracts**
- ❑ **Our method allows for protein recovery with high reproducibility and minimal level of protein fragmentation or non-biological PTMs**
- ❑ **The extracts are provided in two ready-to-use alternative formats**
  - ❑ ***Pre-digested extracts (tryptic peptides) serve the need of instrument performance monitoring***
  - ❑ ***Intact protein extracts are designed for sample preparation method development and optimization***
- ❑ **The extracts provide reference material for comprehensive LC/MS instrument validation, performance monitoring and method development. They can also be used as a model test material for optimizing protein mass spec sample preparation.**