Yeast and human protein extracts for mass spectrometry method development and instrument validation

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1. Introduction

To test protein mass spec sample preparation procedures and validate mass spec instrument performance, complex biological samples are required. Total 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*

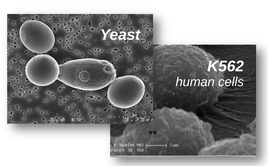
Here we describe total protein extracts from yeast and human cells that meet the above criteria. Two extract formats have been developed:

- *Intact protein extracts; primary use - sample preparation method development and optimization*
- *Pre-digested extracts (peptides); primary use - instrument validation and performance monitoring*

2. The extract description

Source

Yeast extract – from *Saccharomyces cerevisiae*
~6,600 ORFs; all proteins quantified previously.
Human extract – from K562 cells
Complex human proteome with a large dynamic range.



A comparison of alternative extract preparations

Extraction method	**Protein degradation** (% of nonspecific breaks)	**Trypsin inhibition by extraction agent**
Urea	**2%**	**Lack of inhibition** (10% missed cleavages) (Urea was diluted to 1M prior to digestion)
Protease inhibitor cocktail (Roche)	**20%**	**Strong inhibition** (55.9% missed cleavages)
GuCl	Not tested	**Strong inhibition** (44.4% missed cleavages at 0.5M GuCl concentration)

Non-biological PTMs in Urea-extracted proteins

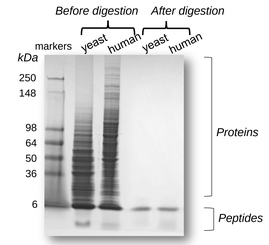
Extract	**Carbamylated peptides**	**Deamidation spectra**	**Oxidation spectra**
Yeast	0.12%	8%	2%
Human	0.15%	4.6%	0.7%

Carbamylation was prevented by reducing proteins at low temperature (37ºC versus 55ºC-65ºC). The temperature did not affect reduction efficiency.

Urea allowed for extraction of proteins with minimal level of degradation or non-biological PTMs without adverse effects on downstream sample preparation steps.

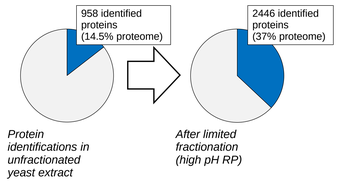
3. Intact extracts for mass spec method development

Suitable for proteolysis



Efficient digestion with trypsin.

Proteome fractionation



Peptide and protein identifications

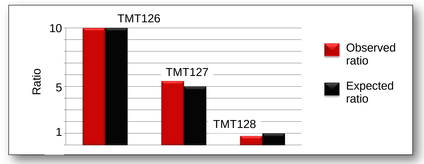
Extract	**Unique peptides**	**Proteins**
Yeast	11924	1525
Human	15576	2075

Q Exactive (Thermo), 2h gradient

Phosphopeptide enrichment

Extract	**Total peptides**	**Phosphopeptides**	**% of phosphopeptides**
Yeast	2502	1715	68.5%
Human	1768	1537	87%

Labeling with mass tags



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

The data show good correlation between observed and expected protein quantitation levels.

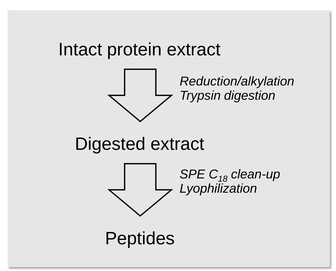
4. Pre-digested extracts for instrument validation

Features

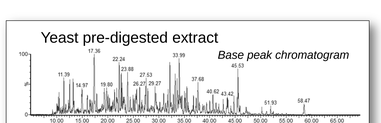
- ✓ Ready for analysis - *no need to reduce, alkylate, digest, or clean-up the sample*
- ✓ Efficiently digested (less than 8% missed cleavages)
- ✓ Highly pure *(SPE C_{18} clean-up)*
- ✓ Lyophilized

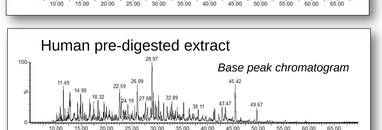
Applications

- ✓ Instrument method optimization and validation
- ✓ Instrument performance monitoring
- ✓ Inter- and intralaboratory benchmarking



Instrument, laboratory and MS 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



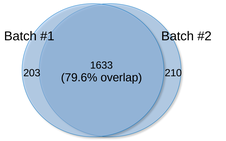


4. Extract reproducibility

Protein and peptide composition reproducibility

Extract	**Protein overlap**		**Peptide overlap**	
	Samples from different lots	**Samples from the same lot**	**Samples from different lots**	**Samples from the same lot**
Yeast	90.5%	90%	79.3%	78.5%
Human	79.6%	79.5%	57.9%	58.1%

Protein Venn diagram
Human extract lots



Quantitation via spectral counting

Yeast extract batches *Spectral counting*			
Protein	#1	#2	#3
YBR118W	358	375	368
YGR192C	378	344	335
YCR012W	369	336	304
YAL005C	307	371	336
YBR196C	153	153	146
YLR249W	150	129	128
YDL145C	23	22	25
YDR261C-D	31	30	28
YKL035W	31	26	32
YOR375C	28	32	28
YDL195W	21	22	21
YDR023W	26	24	23
YHR064C	29	29	28

Human extract batches *Spectral counting*			
Protein	#1	#2	#3
ENOA	137	127	140
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
PPIB	20	17	16
BLVRB	20	21	23
AN32A	21	19	19

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

Protein	**Peptide**	**Batch**	**Normalized abundance***
ABL1_HUMAN	EISDIVOR	A	1.00
		B	1.23
		C	0.99
SHC1_HUMAN	ALDFNTR	A	0.85
		B	1.00
		C	1.10
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*

The extract lots show high reproducibility with respect to protein composition and abundance

5. Conclusions

- ❑ **We developed MS ready total yeast and human protein extracts**
- ❑ **Our method allows for protein recovery with high reproducibility and minimal level of protein degradation or non-biological PTMs**
- ❑ **The extracts are provided in two ready-to-use alternative formats**
  - ❑ ***Intact protein extracts are designed for sample preparation method development and optimization***
  - ❑ ***Pre-digested extracts (peptides) serve to meet the need of instrument validation and performance monitoring***
- ❑ **The extracts show excellent performance in various applications and provide conditions for comprehensive LC/MS instrument validation and method development**