1. Introduction

To monitor mass spec instrument performance for proteomics applications and optimize protein mass spect 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** - Saccharomyces cerevisiae -6,600 ORFs; all proteins quantified previously.

**Yeast extract**

- **Cell lysis**
  - The process is optimized to allow for maximal protein fragmentation.
  - Instant lysis of endogenous proteins to assure virtual lack of non-proteinic cleavages.
  - Only the reagents compatible with LC and MS are used.

**Extract preparation**

- **Sample preparation and digestion**
  - Strict control over the procedural steps assure that non-biological PTMs are maintained at lowest levels.
  - 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 (C18).
  - Peptides are dispersed in 100 uL eluents with high accuracy and lyophilized for long term stability.

**Mass spec analysis report for the extracts**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Human</th>
<th>Yeast</th>
</tr>
</thead>
<tbody>
<tr>
<td>Identified proteins</td>
<td>1970</td>
<td>2768</td>
</tr>
<tr>
<td>Total peptides</td>
<td>17,767</td>
<td>22,258</td>
</tr>
<tr>
<td>Spectrum match</td>
<td>99%</td>
<td>100%</td>
</tr>
<tr>
<td>Unique peptides</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Quantitation spectra</td>
<td>99%</td>
<td>100%</td>
</tr>
<tr>
<td>Oxidation spectra</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Carbamylated spectra</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Nonspecific cleavages</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Missed tryptic cleavages</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>

**Extract purity**

- Yeast extract purity
  - Yeast extract purity
    - Human total protein: 95.64 ng/ug
    - Human total protein: 95.66 ng/ug
  - Yeast extract purity
    - Human total protein: 95.64 ng/ug
    - Human total protein: 95.66 ng/ug

**Extract recovery**

- Yeast extract recovery
  - Human total protein: 95.64 ng/ug
  - Human total protein: 95.66 ng/ug

**Extract enrichment**

- Yeast extract enrichment
  - Human total protein: 95.64 ng/ug
  - Human total protein: 95.66 ng/ug

**Extract quality**

- Yeast extract quality
  - Human total protein: 95.64 ng/ug
  - Human total protein: 95.66 ng/ug

**Extract stability**

- Yeast extract stability
  - Human total protein: 95.64 ng/ug
  - Human total protein: 95.66 ng/ug

**Extract reproducibility**

- Yeast extract reproducibility
  - Human total protein: 95.64 ng/ug
  - Human total protein: 95.66 ng/ug

**Extract control**

- Yeast extract control
  - Human total protein: 95.64 ng/ug
  - Human total protein: 95.66 ng/ug

**Extract biological content**

- Yeast extract biological content
  - Human total protein: 95.64 ng/ug
  - Human total protein: 95.66 ng/ug

**Extract applications**

- Yeast extract applications
  - Human total protein: 95.64 ng/ug
  - Human total protein: 95.66 ng/ug

3. Pre-digested extracts

**Reference material for instrument performance monitoring**

- Human pre-digested extract
  - Human pre-digested extract
    - Human total protein: 95.64 ng/ug
    - Human total protein: 95.66 ng/ug
  - Human pre-digested extract
    - Human total protein: 95.64 ng/ug
    - Human total protein: 95.66 ng/ug

**Test results by different users**

- Human pre-digested extract
  - Human total protein: 95.64 ng/ug
  - Human total protein: 95.66 ng/ug

4. Intact extracts

**Test material for optimization of sample preparation**

- Yeast extract digest
  - Yeast extract digest
    - Yeast total protein: 95.64 ng/ug
    - Yeast total protein: 95.66 ng/ug
  - Yeast extract digest
    - Yeast total protein: 95.64 ng/ug
    - Yeast total protein: 95.66 ng/ug

**Optimizing non-tandem based protein quantitation**

- Yeast extract digest
  - Yeast total protein: 95.64 ng/ug
  - Yeast total protein: 95.66 ng/ug

**Establishing a method for fractionation of a complex peptide mix**

- Yeast extract digest
  - Yeast total protein: 95.64 ng/ug
  - Yeast total protein: 95.66 ng/ug

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 spect sample preparation.