TECHNICAL MANUAL

MS Compatible Yeast and Human Protein Extracts

Instructions for Use of Products
V6941, V6951, V7341 and V7461

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All technical literature is available at: www.promega.com/protocols/
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E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

1. Description

The complexity of biological samples places high demand on mass spec analytical capability. Adequate monitoring of instrument performance for proteomics studies requires equally complex reference material such as whole-cell extracts. However, whole-cell extracts available commercially are developed for general research (e.g., enzymatic or Western blot analysis) and contain detergents and salts that interfere with reverse phase liquid chromatography and mass spectrometry. Even after clean up, the extracts need to be digested, requiring time, labor and experience to generate samples for use in mass spectrometry.

To address the need for complex protein material, we have developed whole-cell protein extracts from yeast and human cells. The yeast extract offers the convenience of a relatively small and well annotated proteome, whereas the human extract provides a complex proteome with large dynamic range. The human extract also serves as reference material for studies targeting the human proteome. The extracts are free of compounds that interfere with reverse phase liquid chromatography-mass spectrometry (LC-MS), and have been reduced with DTT and alkylated with iodoacetamide then digested with Trypsin/Lys-C Mix and lyophilized. These digested extracts (tryptic peptides) can be readily reconstituted in trifluoroacetic acid (TFA) or formic acid and injected into an instrument.

The same human and yeast whole-cell extracts also are provided in an intact (undigested) form for users who would like to develop an independent method for preparing protein mass spectrometry samples. For convenience, the intact extracts are provided as a frozen solution.

1. Description (continued)

Consistent extract protein composition is ensured by tight control over cell culture conditions and manufacturing process. Lot-to-lot consistency of extracts is monitored by various protein and peptide qualitative and quantitation methods, including LC-MS. (Quality control results are provided upon request.) Our manufacturing process assures compatibility with reverse phase liquid chromatography and mass spectrometry, minimal nonspecific protein fragmentation, nonbiological post-translational modifications and, for digested extracts, minimal undigested peptides. The extracts are optimized for a high number of peptide and protein identifications in mass spectrometry analysis.

We recommend these extracts as reference material for mass spec instrument performance monitoring, method development and laboratory-to-laboratory instrument performance standardization.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
MS Compatible Yeast Protein Extract, Digest	**100µg**	**V7461**

This product is a whole-cell protein extract prepared from *Saccharomyces cerevisiae* cells, digested and supplied lyophilized. Includes:

- 100µg MS Compatible Yeast Protein Extract, Digest

PRODUCT	SIZE	CAT.#
MS Compatible Human Protein Extract, Digest	**100µg**	**V6951**

This product is a whole-cell protein extract prepared from human K562 cells, digested and supplied lyophilized. Includes:

- 100µg MS Compatible Human Protein Extract, Digest

PRODUCT	SIZE	CAT.#
MS Compatible Yeast Protein Extract, Intact	**1mg**	**V7341**

This product is a whole-cell protein extract prepared from *Saccharomyces cerevisiae* cells and supplied as a frozen solution. Includes:

- 1mg MS Compatible Yeast Protein Extract, Intact

PRODUCT	SIZE	CAT.#
MS Compatible Human Protein Extract, Intact	**1mg**	**V6941**

This product is a whole-cell protein extract prepared from human K562 cells and supplied as a frozen solution. Includes:

- 1mg MS Compatible Human Protein Extract, Intact

Storage Conditions:

MS Compatible Yeast and Human Protein Extracts, Digest (tryptic peptides): Store lyophilized digests at –20°C. Prior to use, reconstitute the lyophilized digests in the desired solution (e.g., 0.1% TFA or formic acid). Store the reconstituted extract at 4°C for up to 3 weeks or at –20°C or –70°C for up to 6 months. To retain maximum stability, do not freeze and thaw more than twice.

MS Compatible Yeast and Human Protein Extracts, Intact (undigested extracts): Store the frozen intact extracts at –70°C. To retain maximum stability, do not freeze and thaw more than twice.

3. Recommendations for Handling the Extracts

3.A. Reconstitution of Digested Extracts (Cat.# V6951 and V7461)

The MS Compatible Yeast and Human Protein Extracts, Digest, were reduced with DTT and alkylated with iodoacetamide then digested with Trypsin/Lys-C Mix and cleaned up with solid phase extraction, and are supplied lyophilized. The following text includes instructions on extract reconstitution and use.

Materials to Be Supplied by the User

- 0.1% TFA or formic acid

1. Add 100µl of 0.1% TFA or formic acid to a vial of lyophilized digested extract. Reconstitute by gentle swirling or pipetting the solution for 20–30 seconds.
2. Inject 1µl (1µg) of peptide mix into the instrument, and analyze.
3. Divide the remaining solution into aliquots and snap freeze on dry ice. Store the reconstituted extract at 4°C for up to 3 weeks or at –20°C or –70°C for up to 6 months.

3.B. Example Mass Spectrometry Sample Preparation Protocol for Intact (Undigested) Extracts (Cat.# V6941 and V7341)

The MS Compatible Yeast and Human Protein Extracts, Intact, are solubilized in 6.5M urea/50mM Tris-HCl (pH 8) at a protein concentration of 10mg/ml, and supplied as a frozen solution.

Materials to Be Supplied by the User

- NANOpure® water
- 0.5M dithiothreitol (DTT)
- 1M iodoacetamide (IAA)
- 50mM Tris-HCl (pH 8)
- trypsin
- trifluoretic acid (TFA)
- Optional: C18 minicolumn

Note: The following protocol describes digestion of the entire volume of intact extract. Adjust the reagent volumes accordingly if only digesting an aliquot of the extract.

3.B. Example Mass Spectrometry Sample Preparation Protocol for Intact (Undigested) Extracts (Cat.# V6941 and V7341; continued)

Reduction

1. Thaw the intact extract at room temperature.
2. Add 1µl of 0.5M DTT (to a final concentration of 5mM), and mix.
3. Incubate at 37°C for 30 minutes.

   Note: Avoid high temperatures (e.g., 50–60°C) during the reducing step. High temperatures induce protein carbamylation due to the presence of urea in the extract solution. Proteins in our intact extract are efficiently reduced at 37°C.

Alkylation

4. Add 1.5µl of 1M iodoacetamide (to a final concentration of 15mM), and mix.
5. Incubate at room temperature for 30 minutes in the dark (e.g., place tube in drawer and close, or wrap tube in aluminum foil and place inside tightly closed box).

Digestion

6. Add 4–6 volumes of 50mM Tris-HCl (pH 8). Dilution is needed to reduce urea concentration for optimal trypsin activity.

   Note: The protein concentration will decrease from 10mg/ml to 1.7–2.5mg/ml when diluted.

8. Add trypsin to the desired trypsin:protein ratio (typically 1:50), and incubate overnight at 37°C.

   Note: To achieve the most efficient digestion, use the Trypsin/Lys-C Mix, Mass Spec Grade (Cat.# V5071). See the *Trypsin/Lys-C Mix Mass Spec Grade Technical Manual* #TM390 for usage information.

9. Acidify sample by adding TFA to a final concentration of 1%, and remove particulate material by centrifuging at 14,000–16,000 × *g* for 10 minutes using a benchtop microcentrifuge. Clean up digested sample using a C18 minicolumn, or inject directly into a mass spectrometry instrument.

   Note: Acidification often results in cloudiness. Be sure to centrifuge the acidified sample.

4. Troubleshooting

Symptom	Cause and Comment
No lyophilized pellet visible in tube of digested extract	The extract pellet was stuck to the lid. Gently tap vial to return pellet to the bottom of vial.

5. Related Products

Product Name	Size	Cat.#
Trypsin/Lys-C Mix, Mass Spec Grade	20µg	V5071
	100µg	V5072
	100µg (5 × 20µg)	V5073
Trypsin Gold, Mass Spectrometry Grade	100µg	V5280
Sequencing Grade Modified Trypsin	100µg (5 × 20µg)	V5111
	100µg	V5117
Sequencing Grade Modified Trypsin, Frozen	100µg (5 × 20µg)	V5113
rLys-C, Mass Spec Grade	15µg	V1671
Arg-C, Sequencing Grade	10µg	V1881
Asp-N, Sequencing Grade	2µg	V1621
Chymotrypsin, Sequencing Grade	25µg	V1061
	100µg (4 × 25µg)	V1062
Glu-C, Sequencing Grade	50µg (5 × 10µg)	V1651
Endoproteinase Lys-C, Sequencing Grade	5µg	V1071
Elastase	5mg	V1891
Thermolysin	25mg	V4001