

YPX™ Yeast Protein Extraction Kit USE AND STORAGE INSTRUCTIONS

INTRODUCTION

The Yeast Protein Extraction Kit is suitable for the solubilization of proteins for a variety of proteomics applications. The kit contains proprietary buffers designed to maximize the extraction of proteins from yeast cells under conditions that are compatible with downstream proteomics applications. YPX Yeast Protein Extraction buffer contains reducing agents and SDS and provides unbiased extraction of proteins from cells. The resulting lysates are compatible with protein quantification assays, fractionation using the Gelfree® 8100 Sample Fractionation System, other SDS-PAGE separations, and the Filter-Aided Sample Preparation (FASP) method.

USAGE GUIDELINES

- YPX Yeast Protein Extraction Kit provides efficient protein extraction for both freshly harvested cells and frozen cells.
- For best results, cells should be harvested during log-phase growth. The conditions of culture can dramatically affect cell density. For consistency, the YPX Yeast Protein Extraction Kit protocol specifies extraction buffer volumes according to wet cell pellet mass.
- The YPX Yeast Protein Extraction Kit contains SDS and reducing agents. For accurate determination of total protein concentration, use an assay that is compatible with detergent and reducing agent.
- The YPX Universal Protein Kit is designed for maximum, unbiased extraction, reduction, and denaturation of whole proteomes. It should not be used prior to functional protein assays.
- For Research Use Only.

STORAGE AND STABILITY

Store YPX Yeast Protein Extraction Kit reagents at 4 °C. Product shelf life is six months.

RECOMMENDED PROCEDURE

MATERIALS NEEDED

- YPX Yeast Protein Extraction Buffer A provided with kit.
- YPX Yeast Protein Extraction Buffer B provided with kit.
- Protease inhibitor cocktail
- Centrifuge tubes
- Vortex/Shaker
- Centrifuge
- Water bath or hot plate set to 100 °C.

PROTOCOL

When preparing cells for proteomics analysis, it is recommended that the YPX Yeast Protein Extraction Kit be used in conjunction with protease inhibitors.

1. Harvest yeast cells from culture by centrifugation at 5000 *g* for five minutes at 4 °C. Remove and discard the supernatant.
2. Add 1 mL YPX Buffer A per 0.3 g wet cell pellet.
3. Vortex the sample to disperse yeast completely in solution.
4. Incubate cells in YPX Buffer A for five minutes at room temperature.
5. Centrifuge incubated cell preparation at 5000 *g* for five minutes. Remove the supernatant completely by aspiration using a pipet. Discard the supernatant.
6. Measure out a volume of YPX Buffer B equivalent to the volume of YPX Buffer A used in step 2. Add protease inhibitors to the measured volume of YPX Buffer B according to manufacturers recommendation.
7. Add the YPX Buffer B plus protease inhibitors buffer solution made in step 6 to the cell pellet. Use a volume of the buffer solution equivalent to the volume of YPX Buffer A used in step 2.
8. Vortex the sample to disperse yeast completely in solution.
9. Incubate the sample in a 100 °C water bath for three minutes.
10. Remove sample from the water bath and allow to cool at 4 °C for 10 minutes.
11. Centrifuge the cooled sample at 20,000 *g* for 10 minutes to pellet the cellular debris.
12. Collect the supernatant for analysis.

REORDERING INFORMATION

To order the YPX Yeast Protein Extraction Kit, visit www.proteindiscovery.com to request a quote, or contact Protein Discovery, Inc. by phone, fax, or e-mail.

(T) 865.521.7400 / (TF) 866.670.9038 / (F) 865.521.3548
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DESCRIPTION

YPX Yeast Protein Extraction Kit.
Contains 50 mL each of YPX Extraction Buffers A and B

PART NUMBER

44102