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A Geno Technology, Inc. (USA) brand name

FOCUS™ Yeast Proteome

(Cat. #786-257)

INTRODUCTION

FOCUS™ Yeast Proteome kit is specifically designed for yeast research and supplied with yeast specific reagents. This kit extracts and solubilizes nearly all of the proteins from yeast, including membrane proteins. Extraction is based on the gentle lysis of yeast cells with yeast lysis enzyme preparation (*Longlife*™-Zymolyase®) specifically prepared and stabilized for long term use. This kit is provided with an optional protocol for the formation of spheroplast and removal of lytic enzyme (Zymolyase®) prior to lysis and extraction of yeast proteins. It is supplied with a proprietary and strong chaotropic extraction buffer to solubilize even the most difficult proteins, suitable for IEF & 2D gel electrophoresis.

ITEM(S) SUPPLIED (Cat. # 786-257)

Description	Size
Yeast Suspension Buffer	15ml
Longlife™ Zymolyase® (1500 Units/ml)	2 x 0.5ml
FOCUS™ Protein Solubilization Buffer [FPS Buffer]	25g
DILUENT- III	30ml

STORAGE CONDITION

The kit is shipped at ambient temperature. Upon arrival store the kit components as individually marked. Stable for 1 year when stored and used as recommended.

ADDITIONAL ITEM(S) REQUIRED

Sonicator, centrifuge, centrifuge tubes, glass beads, reducing agent, carrier ampholyte, and protease inhibitor cocktail.

PREPARATION BEFORE USE

The kit is supplied with FPS Buffer. Allow the FPS Buffer to warm to room temperature before opening the bottle. Read the instructions on the bottles carefully before use. Just before use, hydrate an appropriate amount of the FPS Buffer with DILUENT-III. Add needed agents such as reducing agent, carrier ampholyte, and if necessary an appropriate amount of protease inhibitor cocktail (ProteaseArrest™, Cat. # 786-108).

PROTOCOLS

A. Longlife™ Zymolyase® Assisted Extraction

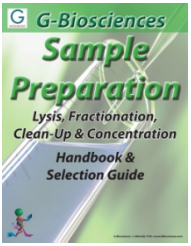
1. Suspend yeast cell pellet in an equal volume of Yeast Suspension Buffer. Add 1µl of β-mercaptoethanol per 100µl of yeast suspension.
2. Vortex until the cell suspension becomes homogenous.
3. Flick the vial containing *Longlife™* Zymolyase® to mix the suspension. Add 10µl of Zymolyase® per 100µl of yeast cell pellet. Gently mix the content.
4. Incubate the cell suspension at 37°C for 30-60 minutes.
5. At the end of incubation, centrifuge the suspension at 10,000x g for 5 minutes. Remove and discard the supernatant carefully, leaving the spheroplast pellet in the tube.
   Optional: *Add 5-10 volume of the Yeast Suspension Buffer to the spheroplast pellet. Resuspend the spheroplast by gently tapping the tube. Centrifuge again as above and discard the supernatant.*
6. Add freshly prepared FPS Buffer in 5-10 times the volume of the spheroplast pellet. Sonicate the suspension with an ultrasonic probe to break down the cell membrane and the genomic DNA. Sonication should be performed in an ice cold bath and during sonication care must be taken to prevent heating. Sonication should be performed with bursts of 20-30 seconds and chill the suspension between ultra-sonic bursts.
7. Centrifuge at 20,000x g for 30 minutes at 20°C and collect clear lysate.
   Optional: *Suspend the residual cell debris in 1/4 the volume of FPS Buffer used in the previous Step-6. Sonicate the suspension once briefly. Repeat Step 7. Collect the extract and pool with the first extract supernatant. Store the yeast protein extract at -70°C until used.*
8. Determine protein concentration. We recommend Non-Interfering Protein Assay, (Cat. # 786-005).
9. Make an appropriate dilution in FPS Buffer before running IEF/2D gels.
   Note: *Proteins solubilized in FPS Buffer that contain CHAPS may not suitable for running SDS-page electrophoresis. Use PAGE-Perfect (G-Biosciences Cat #786-123) to remove detergent before running SDS-page electrophoresis.*

B. Sonication & Glass beads Assisted Extraction

1. Suspend clean yeast cell pellet in 5-10 times volume of prepared FPS Buffer. Add 5 times volume of glass beads (0.5 mm diameter). Sonicate the suspension with an ultrasonic probe to break the cells and break down the genomic DNA. Sonication should be performed in cold (ice cold bath) and during sonication care must be taken to prevent heating. Sonication should be performed with bursts of 30-40 seconds and chill the suspension between ultra-sonic bursts. It may take 10 - 30 minutes depending on the sample size and the sonication strength. Other mechanical devices and grinders may be used instead of ultrasonic probe.
2. Centrifuge the homogenate at 20,000x g for 30 minutes at 20°C to pellet the debris and collect the clear lysate.
   Optional: *Suspend the residual cell debris in 1/4 the volume of FPS Buffer used in the previous Step-1. Sonicate the suspension once briefly. Repeat Step 2. Collect the extract and pool with the first extract supernatant. Store total protein extract at -70°C until used.*
3. Determine protein concentration. We recommend Non-Interfering Protein Assay, (Cat. # 786-005).
4. Make an appropriate dilution in FPS Buffer before running IEF/2D gels.
   Note: *Proteins solubilized in FPS Buffer that contain CHAPS may not suitable for running SDS-page electrophoresis. Use PAGE-Perfect (G-Biosciences Cat #786-123) to remove detergent before running SDS-page electrophoresis.*

RELATED PRODUCTS

Download our Sample Preparation Handbook.



http://info.gbiosciences.com/complete-protein-sample-preparation-handbook

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