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Product Information

IsoYeast Media

Catalog Number **772712, 772704, and 772690**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Stable isotope enrichment of proteins is necessary for study by NMR spectroscopy. Uniform isotope labeling is the biosynthetic labeling with stable isotopes (^{13}C, ^{15}N, and/or D) of all the respective sites in a protein. One way this this can be accomplished is using a methylotrophic yeast (*Pichia pastoris)* expression system for proteins. The yeast are grown in a defined medium with D-glucose or glycerol as the sole sources of carbon in the growth phase, replaced by methanol in the expression phase.

The use of the patented IsoYeast formulation, which includes isotopically labeled carbon and nitrogen sources as well as several other key chemical components, can improve the expression levels of the targeted proteins versus other defined, rich media.

The media are compatible with a variety of carbon and nitrogen sources for labeling. Some examples include glucose, glycerol, ammonium sulfate, and ammonium chloride. Addition of these exogenous components during the preparation of the media opens up avenues for a variety of labeling combinations of ^{15}N, ^{13}C, and D as well as fractional labeling of proteins (e.g., 20% ^{13}C random labeling and 50% fractional deuteration), in a manner similar to *E. coli*. The media are also compatible with inclusion of various amino acids for selective amino acid labeling. No other yeast media currently offer this flexibility.

Components

Each IsoYeast product is a complete system used to prepare dual media – 1 liter of Growth Medium and 250 mL of Expression Medium. This is necessary as the growth phase requires glucose/glycerol, while expression phase requires methanol for induction. Yeast cells have to be transferred from the Growth Medium to the Expression Medium once they have reached the appropriate density.

The dual media are offered as lyophilized powdered components and need addition of only sterile water to the Growth Medium, and water plus methanol to the Expression Medium.

Labeled IsoYeast media may contain a single or multiple stable isotope(s).

Catalog Number	Growth Medium	Isotopic Purity
772690	IsoYeast Powder	Natural Abundance
772712	IsoYeast-^{13}C, ^{15}N Powder	99 atom % ^{13}C, 98 atom % ^{15}N
772704	IsoYeast-^{15}N Powder	98 atom % ^{15}N

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store IsoYeast media at 2–8 °C.

Preparation Instructions

Growth Medium Preparation (1 L)

1. Reagent A – Pour 50 mL of ultrapure water into the bottle labeled Reagent A and shake well for a couple of minutes. Pour contents into a 1 L autoclavable bottle. Repeat this process 2 times to ensure all of Reagent A is removed from the bottle. Add enough ultrapure water to bring the volume up to 600 mL. Sterilize the 1 L bottle containing 600 mL of Reagent A mixture by autoclaving (~30 minute cycle) and let it cool to room temperature. Mix well.
   Note: Precipitate in the mixture at this point is normal and will dissolve during the cell growth.

2. Reagent B – Pour Reagent B from the packet and dissolve in 400 mL of **sterile** ultrapure water in another 1 L bottle. Mix well.
   Note: **Do not heat sterilize** this solution.

3. Add 600 mL of sterilized Reagent A and 400 mL of sterile Reagent B to a 2.8 L baffled flask. Mix well.

4. Reagent E – Shake vigorously to resuspend the precipitate uniformly.

5. Add 10 mL of Reagent E to the baffled flask containing Reagents A and B, along with appropriate antibiotic, if any.
   Note: Precipitate in Reagent E at this point is normal and will dissolve during the cell growth.

Expression Medium Preparation (250 mL)

1. Reagent C – Pour 30 mL of ultrapure water into the bottle labeled Reagent C and shake well for a couple of minutes. Pour contents into a 500 mL autoclavable bottle. Repeat this process 2 times to ensure all of Reagent C is removed from the bottle. Add enough ultrapure water to bring the volume up to 150 mL. Sterilize the 500 mL bottle containing 150 mL of Reagent C mixture by autoclaving (~30 minute cycle) and let it cool to room temperature. Mix well.
   Note: Precipitate in the mixture at this point is normal and will dissolve during the cell growth.

2. Reagent D – Pour Reagent D from the packet and dissolve in 100 mL of **sterile** ultrapure water in another 250 mL bottle. Mix well.
   Note: **Do not heat sterilize** this solution.

3. Add 150 mL of sterilized Reagent C and 100 mL of sterile Reagent D to 2.8 L baffled flask. Mix well.

4. Reagent E - Shake vigorously to resuspend the precipitate uniformly.

5. Add 2.5 mL of Reagent E to the baffled flask containing Reagents C and D, along with appropriate antibiotic, if any, and desired concentration of methanol for induction.
   Note: Precipitate in Reagent E at this point is normal and will dissolve during the cell growth.

Procedure

Culture of Yeast

1. Inoculate the ~1 liter of prepared Growth Medium with 10 mL of a seed culture of yeast cells from an overnight growth.

2. Grow the culture at 30 °C to an OD_{600} = 8–10 (20–24 hours) with shaking at 200 rpm.

3. Centrifuge the culture at 3,000 rpm for 10 minutes.

4. Discard the Growth Medium and resuspend the cell pellet in 250 mL of Expression Medium.

5. Add methanol (~1.88 mL) to a final concentration of 0.75% (v/v) and incubate the culture at 28 °C with shaking at 200 rpm.

6. Replenish methanol (~1.25 mL) to a concentration of 0.5% (v/v) every 12 hours and continue shaking for 3–4 days. Save aliquot from culture every 24 hours for SDS-PAGE gel to determine time of optimal protein expression before harvesting.

7. Protein will be secreted into medium if cloned into yeast vector with alpha factor secretion signal or expressed within cells when no secretion signal is present.

Results

Three different proteins, Interleukin-8 (IL-8), Human Serum Albumin (HSA), and human glycoprotein CD14 were expressed in *Pichia pastoris* grown under the following conditions:[1]

- Yeast grown in a commercially available rich medium (growth) and secreted in rich BMMY medium (expression)

- Yeast grown on IsoYeast media (Growth and Expression) under identical conditions.

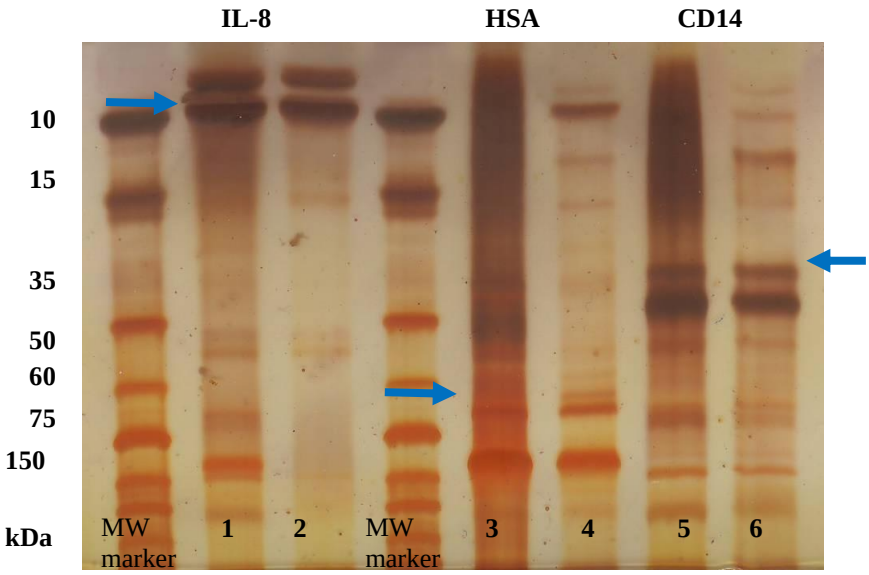
A SDS-PAGE gel was run on each secreted sample without purification (see Figure 1). The bands for the respective proteins are marked with arrows on the gel. Molecular masses of the protein are: IL-8 (9 kDa), CD14 (32 kDa), and HSA (68 kDa).

The gel also shows IsoYeast media have much cleaner expression of the secreted proteins (see Figure 1), easing subsequent purification.

References

1. Data courtesy of Dr. Tom Masi and Dr. Nitin Jain, University of Tennessee

Figure 1.
Expression of 3 Different Proteins in *Pichia pastoris*



Lane 1: IL-8 in commercial media
Lane 2: IL-8 in IsoYeast media
Lane 3: HSA in commercial media
Lane 4: HSA in IsoYeast media
Lane 5: CD14 in commercial media
Lane 6: CD14 in IsoYeast media

Total yields of protein in the 50 mL shake-flask cultures are comparable in the rich and IsoYeast media, and ranged from 0.5–2 mg. The yields in fermentation cultures ranged from 3–10 mg (data not shown). All proteins were fully active as judged by their specific functional assays.