



EnPresso Y Defined



EnPresso Y Defined (Y22001)

Components	EnPresso Y Defined Each bag contains three tablets composed of minerals, vitamins and trace elements, inorganic and organic nitrogen for pH control and polysaccharide substrate	40 bags
	Reagent A (3000 U/L) Glucose-releasing agent	20 mL
Format	Each bag contains tablets sufficient for a 50 mL culture. All components are from animal-free sources. Tablets are manufactured using standard aseptic techniques and filtration or gamma irradiation to ensure sterility.	
Storage	Store kit contents at 4-25°C. After opening, store Reagent A at 4-8°C.	
Shelf life	Expiration date is indicated on the box.	

Additional items needed

- Standard yeast medium (1 mL) for pre-culture
- Sterile shake flask, 500 mL or multiwell plates
- Sterile water, 50 mL
- Inducer: methanol
- Anti-foaming agent such as AntiFoam 204, Sigma Aldrich, when using Ultra Yield Flasks or other baffled flasks.

Note: For shake flask cultures, we recommend the use of Ultra Yield Shake Flasks with AirOtop Enhanced Seals to improve aeration, especially in large volume cultures.

Description

EnPresso® Y Defined is a pre-sterilized growth system designed to increase the yield of functional proteins expressed in *Pichia pastoris* when using AOX promoters. The system provides stress-free conditions, faster growth and eliminates the need for time-consuming media changes required when using conventional methanol-induction. EnPresso growth systems provide optimal conditions for growth, metabolism and protein expression in microbial cultures. Protein yields are increased by enabling cultures to reach far higher cell densities than those achieved using conventional media.

By controlling growth rate and metabolism, a greater proportion of expressed protein can be correctly folded to improve solubility, minimize the risk of inclusion body formation, and ensure functionality of the final product.

EnPresso growth systems maintain pH, provide adequate minerals, vitamins and trace elements to support growth, and use proprietary EnBase® technology to ensure a constant, slow release of glucose from a polysaccharide substrate.

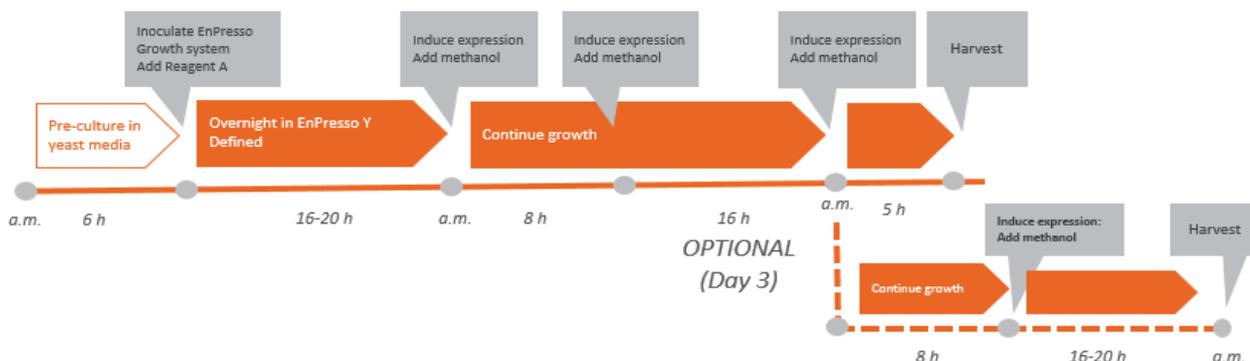
Recommended conditions

- Culture volume:** 10% of flask volume (20% only if using Ultra Yield Flasks)
- Shaking:** 250 rpm, 25-50 mm amplitude. Use 25 mm amplitude for Ultra Yield Flasks
- Temperature pre-culture:** 30°C
- Temperature culture:** 28-30°C

Detailed protocol overleaf →



Protocol for shake flask cultures (50 mL) - optimized for *Pichia pastoris* using an AOX promoter



EnPresso Y Defined growth system - from pre-culture to harvest within 3 days

Day 1

1. Prepare an inoculum: inoculate 1 mL of standard yeast medium with a single colony from an agar plate or from a glycerol stock.
2. Incubate at 30°C for 6 h with vigorous shaking.
3. Add contents of one orange bag (3 tablets) to 50 mL of sterile water in a sterile 500 mL shake flask. When using auxotrophic yeast strains, add appropriate amino acids to the medium.
4. Inoculate with the pre-culture inoculum (1:100 of culture volume).

Note: For best results, take the inoculum from an actively growing culture rather than an overnight culture, with an inoculation cell density of OD₆₀₀ 0.1-0.2.

5. Add 25 µL Reagent A (final concentration 1.5 U/L).

Recommendation: Add anti-foaming agent, such as 5 µL AntiFoam 204 per 50 mL culture volume.

6. Close the flask securely with an air-permeable closure (DO NOT USE ALUMINUM FOIL).
7. Incubate culture at 28-30°C, 250 rpm overnight (16-20 h).

Day 2

8. Add the induction agent (0.25 mL methanol).
9. Continue incubation at 28 -30°C, 250 rpm.
10. After 8 hours, add 0.5 mL methanol. Continue incubation overnight (16 h).

Day 3

11. Add 0.25 mL methanol.
12. a) Incubate for further 5 hours, then harvest.
OPTIONAL
b) Incubate for further 8 hours, then add 0.5 mL methanol. Cultivate overnight (16-20 h) and then harvest on day 4.

Note: This standard protocol has been optimized to enhance the growth of *Pichia pastoris* cultures using AOX promoters. For tips and hints on how to further optimize methanol and Reagent A dosing if needed and how to achieve optimal performance when growing other strains or using alternative promoters, visit www.biosilta.com.

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