



EnPresso® B Animal-free 500



EnPresso® B Animal-free 500 (B11151)

Components	Tablets, silver bag:	2 bags
	Each bag contains 20 tablets composed of minerals, vitamins and trace elements, inorganic and organic nitrogen for pH control and polysaccharide substrate.	
	Booster 500	2 jars
	Each jar contains a powder composed of extra nutrients and polysaccharide substrate.	
	Reagent A (3000 U/L)	5 mL
	Glucose-releasing agent.	
Format	Each bag and jar contains sufficient components for a 500 mL culture. Tablets, Booster 500 powder and Reagent A are manufactured using standard aseptic techniques and filtration or gamma irradiation to ensure sterility.	
Storage	Store unopened 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

- LB medium (20 mL), shake flask for pre-culture
- Sterile shake flask (5 L) or Ultra Yield Flask (2.5 L)
- Sterile water (500 mL)
- Sterile water (50 mL) and a sterile magnet in a sterile bottle for dissolving the Booster 500 powder
- Antibiotics
- Inducer such as IPTG
- Anti-foaming agent, such as AntiFoam 204 when using Ultra Yield Flasks or other baffled (fluted) flasks

Description

EnPresso® B is a pre-sterilized growth system designed to increase the yield of functional protein from *E. coli*-based expression systems. 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: 250-300 rpm, 25-50 mm amplitude. Use 25 mm amplitude for Ultra Yield Flasks.

Temperature pre-culture: 37°C

Temperature culture: 28-30°C

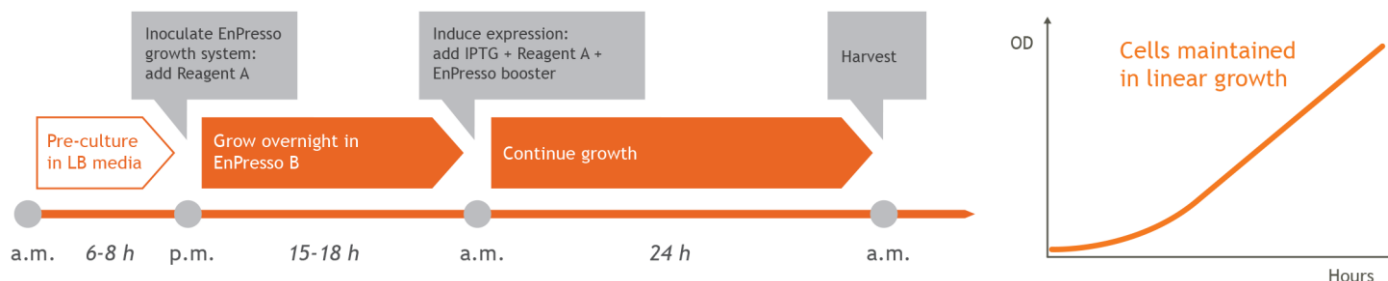
Note: To improve aeration in shake flask cultures, we recommend the use of Ultra Yield Flasks with AirOtop Enhanced Seals. Improved aeration has been shown to further enhance the performance of EnPresso growth systems for bacterial cultures.

IMPORTANT NOTICE:

It is essential to follow EnPresso B protocols in detail. Using a fresh pre-culture, the correct shake speed, air-permeable closures and recommended cultivation times are critical to ensure success.

Caution: Never use aluminum foil or plastic lids during incubation.

Detailed protocols overleaf→



EnPresso B growth system - from pre-culture to harvest within two days

Protocol for shake flasks - 500 mL culture

Day 1 - step 1

1. Prepare inoculum from a glycerol stock or use a single colony grown overnight on an agar plate. Inoculate 20 mL of LB medium containing antibiotics in a shake flask.
2. Incubate at 37°C for 6-8 hours with vigorous shaking.

Optional: If you wish to autoclave the Booster 500 powder before use, dissolve and autoclave it on day 1 to have the Booster 500 ready for use in the morning of day 2 (see point 10 below).

Day 1 - step 2

3. Add contents of one silver bag (20 tablets) to 500 mL of sterile water in a sterile 5 L shake flask.
4. Immediately shake vigorously to ensure tablets begin to dissolve.
5. Add required antibiotics. If using Ultra Yield Flasks, add anti-foaming agent such as 50 µL AntiFoam 204 per 500 mL culture volume.
6. Inoculate with 1:25 of the pre-culture inoculum (20 mL).
7. Add 250 µL Reagent A (final concentration 1.5 U/L)
8. Close the flask securely.
9. Incubate overnight (15-18 h) at 30°C, 250-300 rpm.

Day 2

10. Dissolve the Booster 500 powder in 50 mL sterile water. This can be done by decanting the powder from the jar into a glass bottle containing 50 mL sterile water and a sterile magnet for mixing. Ensure aseptic conditions (laminar flow bench) to keep the Booster 500 sterile. Close the bottle and mix with a magnetic stirrer until the powder has dissolved (10 minutes). Alternatively, the Booster 500 powder can be dissolved under non-aseptic conditions and autoclaved before use. DO NOT dissolve or autoclave the Booster 500 powder in the plastic jar.
11. Add the dissolved Booster 500 to the culture.
12. Add induction agent, and 250 µL Reagent A. If using Ultra Yield Flasks, add 750 µL Reagent A.
13. Continue to incubate at 30°C, 250-300 rpm for a further 24 hours.

Day 3

14. Harvest.

Note: After complete dissolution of EnPresso B tablets, small crystals of magnesium salts may be visible, but these will not affect performance.

Note: For convenience, the dissolved medium and dissolved Booster 500 solution can be autoclaved and used within 2 weeks.

Note: After adding induction agent it may be possible to harvest after 6 hours.

Note: If you expect **toxic target proteins**, please refer to our enhanced protocol at: <http://biosilta.com/products-support/instructionsmsds/>

Note: For tips and hints on how to maximize performance for a specific culture or for handling larger volumes, visit www.biosilta.com or e-mail us at info@biosilta.com

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