



EnBase™ Flo

1L Shake Flask Set

User Manual EBLM100 – Rev. 1.0

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Table of Contents

Table of Contents	1
EnBase™ Flo Shake Flask Set Contents	2
EnBase™ Product Family	3
Theory Behind the EnBase™ Technology	4
About EnBase™ Flo Shake Flask Cultivation	6
EnBase™ Flo Cultivation Procedure	8
Hard Facts	10
Troubleshooting	11
Ordering Information	12
Gel EnBase™ Products	12
Liquid EnBase™ Products	13
Quick Reference Guide	14

EnBase™ Flo Shake Flask Set Contents

EnBase™ Flo Shake Flask Set Components and Storage Conditions

ITEM	CONTENTS	STORAGE
1. Oxygen permeable membrane covers	10 pcs. for cultivation	RT, aseptically packed
2. EnZ I'm (3000 U/L):	1.5 ml, sterile	+4 °C, 3 months
3. EnBase™ Flo Medium	5 x 100 ml of balanced medium	RT, 5 weeks
4. Medium supplement: Thiamine	1 ml, sterile	RT, 3 months
5. EnBase™ Medium for re-suspension	20 ml of medium for re-suspension of inoculum, sterile	RT, 5 weeks
6. Medium booster	50 ml of complex medium additives, sterile	RT, 5 weeks

Storage of the set components: See the best before-date and storage conditions from the packages. RT: Room temperature, 18-25 °C

2. EnZ I'm is stable at room temperature but should be **stored at + 4 °C on arrival.**

3. EnBase™ Medium: After addition of Thiamine (**4.**), use fresh.

5. EnBase™ Medium for re-suspension is not suitable for bacterial cultivation.

Disposal of spare media: The medium components are not harmful in small concentrations and can be discarded without precautions.

EnBase™ Product Family

Gel-based EnBase™ technology is currently available in mini shake flasks, starter tubes, 96-well plates, 24-, 48- and 96-deep well plates.

Liquid EnBase is available in package sizes of 5 x 100 ml and 2 x 500 ml.

Please see the ordering information in this manual.

Detailed scientific info at: www.biosilta.com

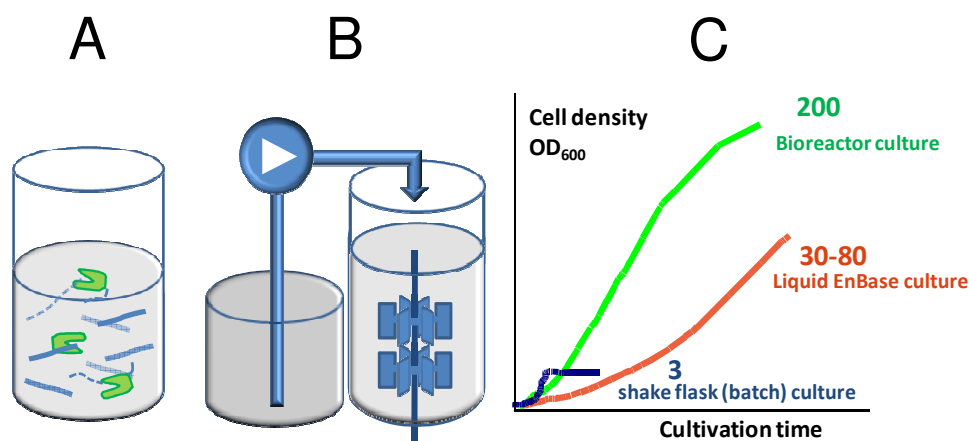
Theory Behind the EnBase™ Technology

Simple shaken cultivations are used worldwide for screening, optimisation, protein production and other cell cultivation purposes. Cultivations are most often performed as batch cultures, because constant nutrient feeding in such a small scale is challenging. Cell yields and product levels in the batch process described above are much lower when compared to cultivation in bioreactors utilising a FED batch cultivation process. In **fed-batch** processes high cell densities can be obtained without oxygen limitation. The specific growth rate of the culture will be lower than in batch cultures and therefore the cultivation time is significantly longer. However, with **fed-batch technology** it is possible to reach cell densities up to OD_{600} of 400 in bioreactors, whereas in shaken batch cultures enough oxygen can be normally delivered only for OD_{600} from 1 to 10. Low protein production in batch culture is caused by a number of factors including excess nutrient supply, non-controlled growth, high respiration rate of bacteria, changed pH during cell growth, and production of growth-limiting compounds due to overflow metabolism or anaerobic conditions.

BioSilta EnBase™ Flo technology opens the way to use fed-batch technology in simple shaken cultures like those performed in shake flasks. In contrast to a typical fed-batch process, where the main substrate feed (mostly glucose) is added by a pump to the bioreactor, in EnBase™ Flo system glucose is released from soluble polymers by the biocatalytic activity of EnZ I'm. The medium compounds from EnBase™ Flo guarantee some basic growth to a very limited level, but high cell density is obtained only by addition of an EnZ I'm.

In EnBase™ Flo cultivations:

- ✓ Slow glucose release controlled by EnZ I'm addition ensures defined cell growth in a fed-batch manner.
- ✓ Oxygen limitation and overflow-metabolism are avoided and thereby optimal conditions for recombinant protein production are provided.
- ✓ Addition of medium boosters and extra dose of EnZ I'm during the recombinant gene expression provide the required biosynthetic activity of bacteria.
- ✓ Balanced use of carbon source (glucose) and peptides (medium boosters) for cell maintenance, growth and protein synthesis is supported.
- ✓ Two-phase cultivation is performed:
 1. The production of high amount of cells (more than 10-fold cell mass as compared to that normally used for induction).
 2. Addition of medium boosters together with the inducer provides enough energy and constituents for efficient recombinant protein synthesis.



Schematic view of the EnBase™ substrate delivery system of EnBase™ Flo system (A) compared to the setup in fed-batch bioreactor (B). The graph (C) shows the typical growth profiles and final cell densities of standard shake flask cultivation (**blue** line), fed-batch process in a bioreactor (**green** line) and EnBase™ Flo cultivation (**red** line).

Benefits of the Liquid EnBase™ technology:

1. You will obtain more recombinant protein, as you can start the induction at a time, where your cultivation has about 10-fold the cell density used in standard batch cultivations.
2. You can use longer expression time (final cell density up to OD₆₀₀ of 40), since the bacteria maintain their metabolic activity and the medium is not quickly spoiled by growth-limiting products.
3. You can use a wide range of cell densities for induction (OD₆₀₀ 5-10 is recommended), which frequently makes OD₆₀₀ measurement for the optimal induction time point unnecessary.
4. Use overnight cultures for induction, making the cultivation user-friendly.
5. You control the cell growth by addition of EnZ I'm and optimise induction and harvest time, which is a benefit compared to the popular lactose/autoinduction system.
6. You can use a ready-made solution for quick setup of cell cultivation and recombinant protein production.
7. EnBase™ Flo is an excellent product for people who:-
 - usually need several litres of culture to obtain enough recombinant protein for purification and analysis
 - need high cell and product yields in a small volume (e.g., enzyme screening, strain testing, protein labelling)
 - need an easy and reliable cultivation system for a high number of parallel cultivations

About EnBase™ Flo Shake Flask Cultivation

Beware!

- ❖ *Growth is linear in balanced growth medium*
 - ✓ *It takes longer time to reach high cell densities than in ordinary batch cultivation, fast is not always the best in this case.*

- ❖ *Shake-flask closure*
 - ✓ *A membrane-cover enables good and safe oxygen transfer. Note however that wetting of any kind of membrane cap results in poor gas exchange and potential failure of the cultivation. In the case of membrane wetting, replace the membrane.*

- ❖ *Shaking speed*
 - ✓ *180 – 200 rpm in shakers with circular orbit of 50 – 25 mm are recommended.*
 - ✓ *Shaking speed depends on the shaker and cultivation shake flask used. Maximise the agitation but avoid wetting the closure.*

Note:

Items needed but not provided:

- Shake flask 1L.
100 ml cultivation volume is 10% of the flask volume which provides good oxygen transfer to the medium.

- Inducer, e.g. IPTG
1.1 ml of 100 mM IPTG solution (0.119 g IPTG + 5 ml distilled H₂O, sterilised by membrane-filtration)

❖ Induction

- ✓ Recommended induction OD_{600} for EnBase™ cultivation is 5-10. For efficient recombinant protein production, we recommend the addition of an extra EnZ l'm and nutrient dose at the time of induction for maximum performance (provided in the shake flask set).
- ✓ EnBase™ Flo provides good conditions for a long induction, therefore expression times from 6 to 24 hours are recommended. To enable correct protein folding and high proportion of soluble protein, a temperature shift (from 30 to 25-30 °C or even lower) can be used after the induction is started.

❖ Harvest

- ✓ Recommended expression time is 6 to 24 h.
- ✓ Induction and harvest times need to be optimised for each specific expression system and customer culture conditions.

Note:

For maximal performance, induction and harvest times need to be optimised for each specific expression system

EnBase™ Flo Cultivation Procedure

The culture is started with a volume of 100 ml. After induction the total volume will be about 110 ml due to addition of medium booster (10 ml) and other medium supplements.

1. Prepare the inoculum:
 - A. Wash the cells with EnBase™ Medium (vial 5.) from LB-plate cultivated overnight at 37 °C.
or
 - B. Inoculate straight from a glycerol stock (minimum OD₆₀₀ of glycerol stock OD₆₀₀ > 10).
or
 - C. Prepare a pre-culture in complex medium (not provided). Calculate needed amount of cells to obtain the start OD₆₀₀ of 0.1-0.2 for main culture. Take the aliquot of needed cells, spin down and remove the supernatant. Re-suspend the cells in small volume of EnBase™ re-suspension medium (vial 5.).
2. Add aseptically to 100 ml of EnBase™ Flo Medium (vial 3.)
 - antibiotics if needed
 - 0.1 ml thiamine (vial 4A)
 - 50 µl of EnZ I'm (vial 2.) until final concentration of 1.5 U/L
3. Pour this into the cultivation shake flask and inoculate. Recommended initial OD₆₀₀ for EnBase™ cultivations is 0.1-0.2.

INFO

- **Inoculum:** The easiest method is to use glycerol stock, but it does not give best cell viability. With all inoculum options it is important to have minimal amount of glycerol /glucose in the EnBase™ growth medium and an initial OD₆₀₀ > 0.1.
- **Recommended EnZ I'm concentration is based on cultivation at 30 °C and pH 7. Under other conditions it is beneficial to optimise the EnZ I'm dosing.**

4. Seal the cultivation flask with a membrane cover (1.).
5. Cultivate bacteria (30 °C, 200 rpm) for 10 to 24 h. During that time the recommended induction cell density of $OD_{600} = 5$ to 10 is reached.
6. Prepare the Booster/Inducer mixture: (can be done in advance)
 - ❖ Take 10 ml of Medium Booster (vial 6.) and add 110 μ l of EnZ l'm (vial 2.) to provide 3 U/L final concentration in 110 ml cultivation volume¹
 - ❖ Add inducer, e.g. 1.1 ml of 100 mM IPTG solution to the Medium Booster (vial 6.)

After (overnight) growth (step 5.), add medium booster/inducer mixture to the cultivation bottle (10 % of the cultivation volume).

7. Continue cultivation at the temperature, which is known to be optimal for production and correct folding of your protein. Harvest after 6 to 24 h expression.

¹ EnZ l'm concentration recommendations: Optimal EnZ l'm concentration is dependent on the cultivation conditions, i.e. oxygen transfer capacity, and also of the bacterial strain and medium. Therefore it may be beneficial to optimise the concentration in the specific conditions to reach best yield.

Hard Facts

TECHNOLOGY

Glucose limited fed-batch. Glucose is released enzymatically from the polymer supplied in the cultivation medium.

SHAKE FLASK AND CULTURE VOLUME

Recommended cultivation volume is 10-20 % of the shake flask volume. Therefore use of 1 liter shake flask, baffled or non-baffled is recommended for this procedure.

ORGANISMS/STRAINS

Applicable strains: W3110, RB791, RV308, BL21 and its DE3 derivatives, Rosetta. Attention: Redox mutants like Origami[™] or Rosetta-gami[™] strains reach lower cell densities due to higher cell maintenance requirements.

MEDIUM

A balanced growth medium is used in the first phase of cell cultivation. For protein production, this medium is supplemented with a booster solution and inducer.

CULTIVATION CONDITIONS

Shaking speed is dependent on the shaker type and flask geometry. For standard 1L shake flasks shaking speed of 180-200 rpm is effective. The cultivation temperature of 30 °C can be used for the overnight culture but after the induction the conditions need to be optimised for each expression system.

REFERENCES

EnBase[™] technology: Panula-Perälä, J., Šiurkus, J., Vasala, A., Wilmanowski, R., Casteleijn, M., Neubauer, P. (2008). Enzyme controlled glucose auto-delivery for high cell density cultivations in microplates and shake flasks. *Microbial Cell Factories* 7, 31.

Troubleshooting

Problem	Cause	Remedy
Long lag-phase	Non-viable cells in the inoculation or low initial cell density	Use fresh cells collected from overnight plate or use pre-culture. Start the EnBase™ cultivation with initial OD ₆₀₀ between 0.05 and 0.2.
Slow start of growth	Glucose accumulation in the beginning of cultivation may cause overflow metabolism and synthesis of growth-inhibiting metabolites.	1. Reduce EnZ I'm concentration 2. Check that the inoculum does not contain glucose.
Poor growth	Medium acidification due to bad oxygen transfer: wetting of closures might be one reason	Prevent medium spilling to the membrane cap by decreasing the shaker speed

Please consult BioSilta (info@biosilta.com) if you are not getting beneficial results in your application. We are here to support you.

Ordering Information

Gel EnBase™ Products

Choose medium: A: EnBase™ complex medium, B: EnBase™-mineral salt medium

EnBase Optimisation Set

EnBase™ Optimisation set	EnBase™ 24 deep-well plate for optimisation, 3 different EnBase™ media, 1 vial EnZ I'm 300 000 units/L, user manual.
EBOS01	1 EnBase™ optimisation set
EBOS02	2 EnBase™ optimisation sets

Shake flask and starter culture

EnBase™ Mini Shake Flask	4 (8) pieces of EnBase™ mini shake flasks, 120 ml (240 ml) EnBase™ medium, 1 vial EnZ I'm 300 000 units/L, user manual.
EBMF04	EnBase™ Mini Shake Flask set, 4 flasks (4 x 20 ml culture)
EBMF08	EnBase™ Mini Shake Flask set, 8 flasks (8 x 20 ml culture)

EnBase™ Starter Tube	6 (12) pieces of EnBase™ Tubes, 120 ml (240 ml) EnBase™ medium, 1 vial EnZ I'm 300 000 units/L, user manual.
EBST12	EnBase™ Starter-Tube set, 12 tubes (12 x 5 ml culture)
EBST24	EnBase™ Starter-Tube set, 12 tubes (24 x 5 ml culture)

EnBase™ Plated Products

EnBase™ 96 Well Plate	6 (12) pieces of EnBase™ 96 micro-well plates, 120 ml (240 ml) of EnBase™ medium, 1 vial EnZ I'm 300 000 units/L, user manual.
EB96S06	EnBase™ 6 plate cultivation set (culture volume 150 µl / well)
EB96S12	EnBase™ 12 plate cultivation set (culture volume 150 µl / well)

EnBase™ 96 Deep-Well Plate	6 pieces of EnBase™ 96 deep-well plates, 120 ml concentrated medium, 1 vial EnZ I'm 300 000 units/L, user manual.
EB96D06	EnBase™ 96DWP, 6 plate set (culture volume 700 µl / well)

EnBase™ 48 Deep-Well Plate	6 pieces of EnBase™ 48 deep-well plates, 120 ml concentrated medium, 1 vial EnZ I'm 300 000 units/L, user manual.
EB48D06	EnBase™ 48DWP, 6 plate set (culture volume 1.5 ml / well)

EnBase™ 24 Deep-Well Plate	6 pieces of EnBase™ 24 deep-well plates, 120 ml concentrated medium, 1 vial EnZ I'm 300 000 units/L, user manual.
EB24D06	EnBase™ 24DWP, 6 plate set (culture volume 3 ml / well)

Liquid EnBase™ Products

EnBase™ 1 L Shake Flask	5 x 100 ml EnBase™ Flo medium, 1 vial EnZ I'm 3000 units/L, medium supplement and booster, membrane covers for shake flasks, user manual.
EBLM100	EnBase™ Flo set for five 1 liter flasks (5 x 100 ml culture)
EnBase™ 2.5 - 5L Shake Flask	2 x 500 ml EnBase™ Flo medium, 1 vial EnZ I'm 3000 units/L, medium supplement and booster, membrane covers for shake flasks, user manual.
EBLM500	EnBase™ Flo set for two 5 liter flask (2 x 500 ml culture)

If you need a specific cultivation format for your research, ask for a custom product and continue to benefit from the EnBase™ technology.

Further information, orders and inquiries:

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Quick Reference Guide

Always study the instructions of this manual carefully before starting cultivation. This *Quick reference guide* can be used as a memory aid when the procedure is familiar.

- ❖ Prepare pre-culture if necessary

1st day:

- ❖ Prepare culture medium
 - ✗ Add thiamine/ antibiotics
- ❖ Inoculate
- ❖ Add EnZ I'm
- ❖ Start cultivation, close the shake flask with membrane

2nd day:

- ❖ Add Inducer/ booster/ EnZ I'm mixture or use cultivation as a pre-culture

3rd day:

- ❖ Harvest



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