



# Application data sheet #01

## Constant temperature incubator shaker **Bio-Shaker BR Series**

### About container shapes, shaking speed, amplitude, and culture efficiency

## Reconsider by examining the factors in shaking culture that tend to be carried out because of conventional behavior and practice in experiments

### Overview

The shaking culture method for microorganisms has many factors. The components of culture mediums and the nature of microorganisms as subjects of culture (optimal culture temperatures, etc.) are not covered in this document, but it is not an exaggeration to say that the efficiency of aeration, which is important when aerobic conditions are required, determines the speed and yield of a culture. When considering aeration, the factors involved are the shape (type) of a culture container, the capacity of a container, the amount of liquid, the shaking method, the shaking speed, and the amplitude. Among these, the shaking method is determined by the shape of the container, while the capacity of the container is decided by the theoretical practices regarding the amount of liquid. The shape of the container, the shaking speed, and the amplitude remain as key variables. In this document, considerations based on experiments are described with these three factors.

### About culture containers

In this study, we investigated the difference of culture efficiency based on the following three glass containers that are frequently used in shaking culture, in addition to the shaking speed and the amplitude.

#### Erlenmeyer flask



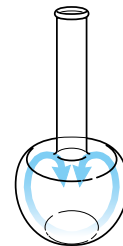
- Commercially available capacity  
50 to 5000ml
- Recommended amount of liquid for shaking culture  
1/5 to 1/3 of the flask capacity
- Shaking method for culture  
Rotary shaking
- Other names  
Conical flask  
Triangular Kolben

#### Erlenmeyer flask with baffles



- Commercially available capacity  
50 to 2000ml
- Recommended amount of liquid for shaking culture  
1/5 to 1/3 of the flask capacity
- Shaking method for culture  
Rotary shaking
- Other names  
Shaking Erlenmeyer flask  
Trypsinizing flask

#### Shake flask



- Commercially available capacity  
50 to 2000ml
- Recommended amount of liquid for shaking culture  
1/5 to 1/2 of the flask capacity
- Shaking method for culture  
Reciprocal shaking
- Other names  
Sakaguchi flask

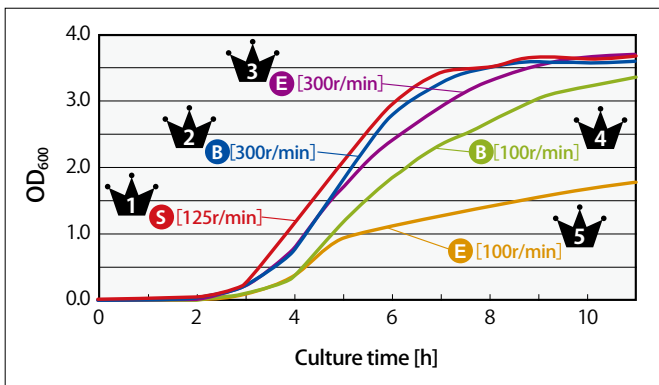
Glass containers are frequently used for shaking culture. This shaking method is rotary shaking. Disposable plastic types are also commercially available. These flasks are often used for bacteria that can easily increase, such as colon bacilli or when culture efficiency does not especially matter. Other than shaking culture applications, glass containers can be strongly shaken in the form of reciprocal shaking, but this is not common because bubbles are generated in the shaking culture.

An Erlenmeyer flask with projections called baffles are located inside. Aeration efficiency is improved as the culture solution is revolved by rotary shaking hits these baffles. However, as the culture solution may form bubbles intensively, this flask should not be used as it is inconvenient. For shaking culture of yeast, this Erlenmeyer flask is recommended to be used with a shake flask as referred on the right section.

This container is unique to Japan and offers high aeration efficiency by adjusting the shaking speed and the amplitude so that the culture solution moves as shown in the figure above. Also, more culture solutions can be contained compared to Erlenmeyer flasks. Disadvantages include the flask height, which is rather high for its capacity, and difficult to clean the insides of the flask. For cultivating molds, the molds may become attached to the bottleneck, so it is necessary to remove them frequently to prevent clogging.

### Results and discussion

#### ① Differences of culture efficiency based on the shape of container (amplitude: 25 mm, *E.coli*)



E = Erlenmeyer flask, B = Erlenmeyer flask with baffles, S = Shake flask  
 1-5 = Culture efficiency order

Constant temperature incubator shaker: BioShaker G-BR-300

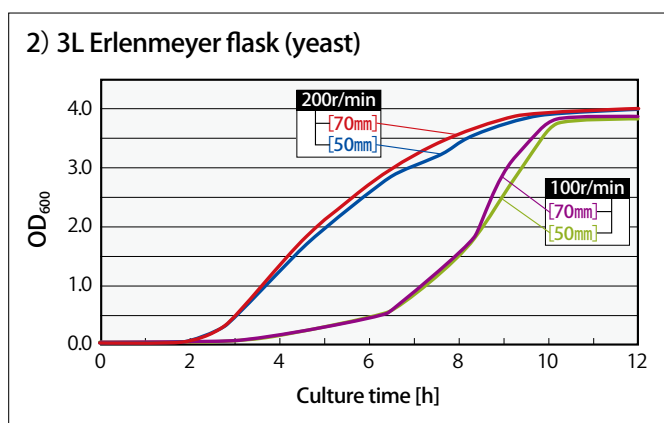
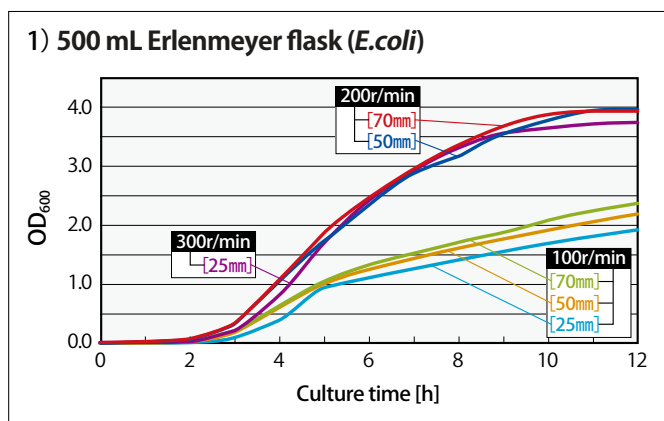
*E.coli*: HB101 strains, 1/1,000 amount of preculture solution is cultivated at +37° C  
 Culture medium/Container capacity: LB culture medium 100 mL in each 500 mL

Indicator of culture efficiency:

High/Low multiplication curve, plotting changes of OD<sub>600</sub> over time

Firstly, the capacity of the container, the amount of liquid, and the amplitude were fixed in order to investigate the differences in the multiplication curves based on the shape of container. The capacity of container was set to 500 mL, which is generally used for shaking culture. The amount of liquid was set to 1/5 the amount of the flask capacity following the theoretical approach. The amplitude was 25 mm, which is the largest in number for commercially available constant temperature incubator shaker. The shaking speed was 100 r/min. and 300 r/min. (a critical speed for general constant incubator shakers) for Erlenmeyer flasks that were rotary-shaken, while shake flasks were shaken in a reciprocal manner at 125 r/min. (only this speed for the above-described liquid movement). Results are shown on the left. The shake flask enjoyed the highest efficiency, followed by the Erlenmeyer flask with baffles. If they can be used, both are likely to provide high efficiency. It also turned out that with a normal Erlenmeyer flask, efficiency comparable to the other two can be obtained if the shaking speed is increased.

## ② Difference in culture efficiency based on the amplitude



Constant temperature incubator shaker:

BioShaker G-BR-300 (amplitude: 25/50 mm)

BioShaker BR-180LF-70RT (amplitude: 70 mm)

*E.coli* : HB101 strains, 1/1,000 amount of preculture solution is cultivated at +37° C

Yeast : S288C strains, 1/100 amount of preculture solution is cultivated at +30° C

Culture medium :

LB culture medium 100 mL (*E.coli*), YPD culture medium 100 mL (yeast)

Container/Shaking method : Erlenmeyer flask (500 mL and 3L), rotary shaking

Indicator of culture efficiency:

High/Low multiplication curve, plotting changes of OD600 over time

We examined the amplitude, which is the subject of frequent discussions.

1) Multiplication curves for rotary shaking at amplitudes of 25 mm, 50 mm, and 70 mm were compared. With 500 mL Erlenmeyer flasks, no conspicuous difference was seen for each amplitude, showing that the effect of shaking speed is greater. (The culture efficiency for 25 mm was lower than that for the other two, but it was found that this could be compensated by increasing the shaking speed.)

2) We established a hypothesis where amplitudes larger than 25 mm would be especially effective in the case of large-capacity containers and large particles of microorganisms. We cultivated yeast that had larger particles than *E.coli* which tends to submerge in 3 L Erlenmeyer flasks in order to compare the amplitudes of 50 mm and 70 mm. The results showed that there were little differences due to the difference in amplitudes, and due to the difference in shaking speeds, there were some differences at the start (Difference in multiplication curves between 100 r/min. and 200 r/min. was large at the early stage, but became almost the same 12 hours later.)

Large amplitudes such as 50 mm and 70 mm are considered to be used to compensate for the shaking speed with large-capacity containers that cannot be shaken at a high speed inevitably, but we could not see any significant difference between 50 mm and 70 mm. Going forward, we plan to compare 25 mm, 50 mm and 70 mm by using 5 L Erlenmeyer flasks, and a shaker flask (reciprocal shaking) that could not be covered in this experiment. Traditionally, in shaking culture of actinomycetes, a reciprocal shaking of shaker flasks at an amplitude of 70 mm is used, and if there are any differences between 50 mm and 70 mm, the shapes of containers and properties of microorganisms could be related.

Now on sale

TAITEC constant temperature incubator shaker offers a lineup supporting 25 mm for small and medium sizes as well as 25mm, 50 mm, and 70 mm for large sizes. For shaking methods, in addition to rotary shaking, reciprocal shaking is effective for shaker flasks that are unique to Japan, while shaking of test tubes and disposable centrifuge tubes are also available. Customers can easily choose from a wide range of products to meet their targets and scales of experiments. For further details, please visit TAITEC-Online.  
<https://e-taitec.com/>



Constant temperature incubator shaker (large-sized)  
BioShaker G-BR-300

Up to twenty-four 500 mL Erlenmeyer flasks. Reciprocal and rotary shaking are switchable. Amplitude is switchable between 25 mm and 50 mm. Shaking speed: 25 to 300 r/min. Operation temperature range is +4° C to 80° C



Constant temperature incubator shaker (large-sized)  
BioShaker BR-180LF-70RT

Up to twenty-four 500 mL Erlenmeyer flasks. Rotary shaking only. Amplitude: 70 mm. Shaking speed: 25 to 250 r/min. Operation temperature range is +4° C to 70° C

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