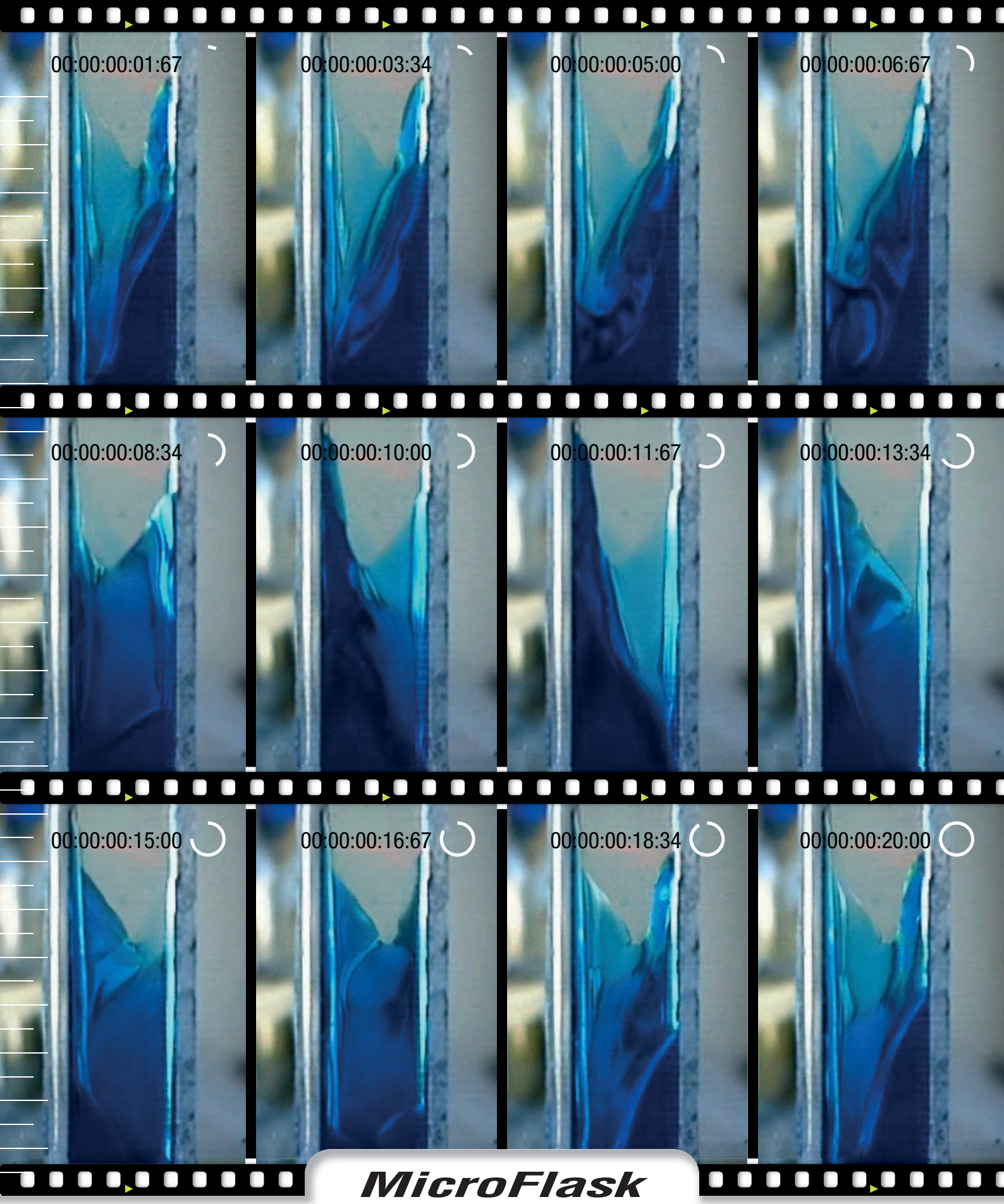



# MicroFlask

***Miniaturize without compromize***

***Simple and low cost bioreactor scale-down to micro-liter volumes***

applikon® BIOTECHNOLOGY

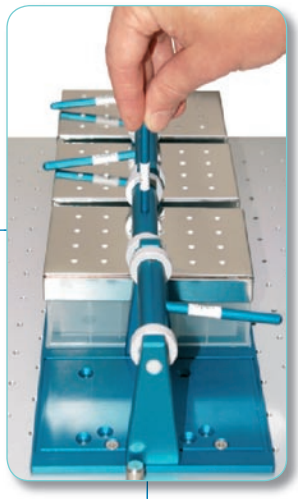
A STEP *AHEAD*

## Introduction

**The MicroFlask is a cultivation system that turns microtiter plates into reliable and easy to operate cultivation systems offering reproducible cultivation results.**

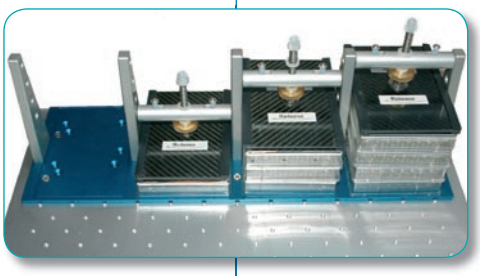
**Miniaturization of cultures to standard 24 or 96 well microtiter plate format is an attractive format because of the common availability of:**

- Low-cost microtiter plates in various shapes and materials
- Robotic equipment for filling wells with growth medium
- Centrifugation (pelleting cells)
- Equipment for UV-VIS or fluorescence measurement, etc.



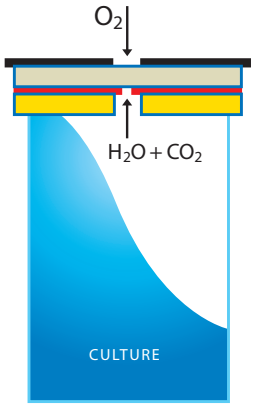
## Main features of the MicroFlask:

- 24 (or 96) single use and low cost bioreactors per titer plate
- Stackable design allows up to 4 plates in one clamp. This results in up to 384 bioreactors on 85 x 125 mm
- Oxygen transfer rates are similar to those in Erlenmeyer flask (40 mmol $O_2$/l/min) and can be achieved with standard orbital shaking equipment
- No well-to-well cross-contamination risk
- Low (and uniform) evaporation rates
- Cell densities of 10 g dry wt/l or more are achievable
- Well-to-well reproducibility
- Sandwich covers converting each well of 24 and 96 well micro-titer plates (both deep-well and low-well plates) into individual “micro-reactors”
- A spring-loaded replicator allows the simultaneous and reproducible inoculation of MicroFlask cultures and sampling from 96 frozen glycerol stocks without thawing the main culture. The stocks can remain viable for years.



## Examples of MicroFlask application areas:

- Discovery of secondary metabolites or new enzyme activities (heterogeneous culture collections)
- Screening and distribution of mutant and construct libraries e.g. in E. coli or yeasts
- Metabolic flux studies and high-throughput screening for high-activity mutants
- Comparative studies, e.g. clinical isolates
- Growth medium optimization for cell lines or production strains

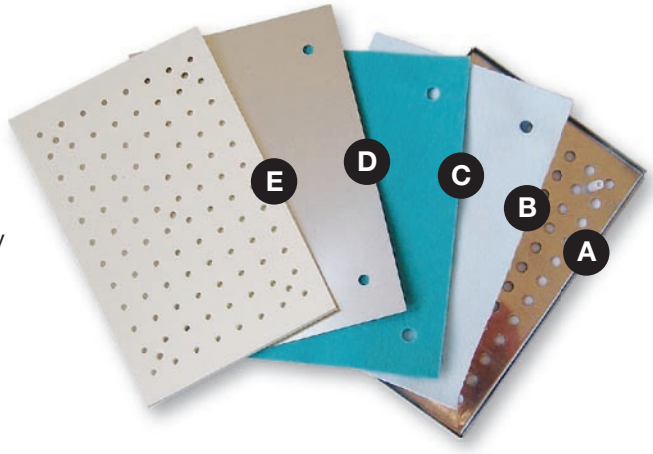



## Oxygen supply

Classic bioreactors for microbial cultures require an air supply of up to two working volumes per minute as an average. The sandwich covers are designed to have a headspace refreshment rate of 1-2 culture volumes per minute.
This headspace refreshment rate secures oxygen concentrations in the headspace of at least 18% (v/v), even at an oxygen consumption rate of 40 mmol $O_2$/L/ minute. At the same time this limits the evaporation to about 2% of the culture volume per day (at an ambient humidity of 50% and 30˚C).

## Sandwich cover

- **A** stainless steel lid for rigid support
- **B** micro fiber layer
- **C** extruded teflon or ePTFE (0.2 µm pores) layer for sterility
- **D** stainless steel foil with pinholes to further limit of evaporation (only in covers for 96-lowwell plates)
- **F** soft silicone layer (to seal the “mini-reactors”)

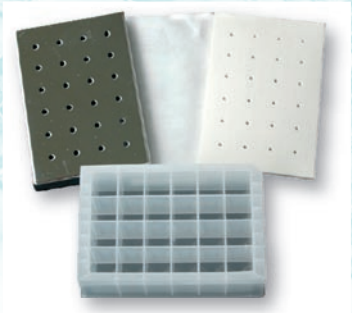



## OTR Specifications MicroFlask-bioreactors

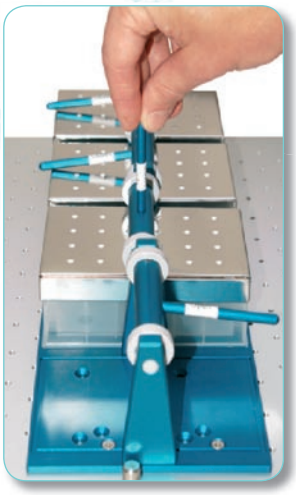
| Type of microtiter plate | Well volume dimension in horizontal plane | Culture volume | Orbital shaking frequency | Shaking amplitude | $O_2$-transfer rate (30˚C, air, 1 bar) | Headspace refreshment rate | Evaporation rate per well (at 30˚C) | Mixing pattern at 300 rpm ampl. 25 mm | Mixing pattern at 300 rpm ampl. 30mm |
|---|---|---|---|---|---|---|---|---|---|
| 24-square deep-well polypropylene, 17x17 mm, depth 40 mm | 11000 µl | 2500 µl<br>2500 µl<br>2500 µl | 300 rpm<br>300 rpm<br>220 rpm | 50 mm<br>25 mm<br>50 mm | 51 mmol $O_2$ / l<br>39 mmol $O_2$ / l<br>35 mmol $O_2$ / l | 2.5 ml / min<br>(1 VVM) | 50% humidity:<br>50 µl $H_2O$ per day |  2500 µl |  2500 µl |
| | | 4000 µl<br>4000 µl | 300 rpm<br>220 rpm | 50 mm<br>25 mm | 24 mmol $O_2$ / l<br>24 mmo $O_2$ / l | 2.5 ml / min<br>(0.6 VVM) | 75% humidity:<br>25 µl $H_2O$ per day | | |
| 24-round low-well polystyrene, ∅ 16 mm, depth 18 mm | 3000 µl | 750 µl<br>750 µl | 300 rpm<br>300 rpm | 50 mm<br>25 mm | 40 mmol $O_2$ / l<br>25 mmol $O_2$ / l | 1.1 ml / min<br>(1.4 VVM) | 50% humidity:<br>30 µl $H_2O$ per day |  1000 µl |  1000 µl |
| | | 1000 µl<br>1000 µl | 300 rpm<br>300 rpm | 50 mm<br>25 mm | 30 mmol $O_2$ / l<br>19 mmol $O_2$ / l | 1.1 ml / min<br>(1.1 VVM) | 75% humidity:<br>15 µl $H_2O$ per day | | |
| 96-square deep-well polypropylene, 8x8 mm, depth 40 mm | 2400 µl | 500 µl<br>500 µl | 300 rpm<br>300 rpm | 50 mm<br>25 mm | 38 mmol $O_2$ / l<br>12 mmol $O_2$ / l | 1 ml / min<br>(2 VVM) | 50% humidity:<br>22 µl $H_2O$ per day |  750 µl |  750 µl |
| | | 750 µl<br>750 µl | 300 rpm<br>300 rpm | 50 mm<br>25 mm | 24 mmol $O_2$ / l<br>7 mmol $O_2$ / l | 1 ml / min<br>(1.3 VVM) | 50% humidity:<br>22 µl $H_2O$ per day | | |
| | | 1000 µl<br>1000 µl | 300 rpm<br>300 rpm | 50 mm<br>25 mm | 18 mmol $O_2$ / l<br>3 mmol $O_2$ / l | 1 ml / min<br>(1 VVM) | 75% humidity:<br>11 µl $H_2O$ per day | | |
| 96-round low-well polypropylene, ∅ 6.5 mm, depth 10 mm | 380 µl | 100 µl<br>100 µl | 300 rpm<br>300 rpm | 50 mm<br>25 mm | 39 mmol $O_2$ / l<br>20 mmol $O_2$ / l | 250 µl / min<br>(2.5 VVM) | 50% humidity:<br>6 µl $H_2O$ per day |  150 µl |  150 µl |
| | | 150 µl<br>150 µl | 300 rpm<br>300 rpm | 50 mm<br>25 mm | 32 mmol $O_2$ / l<br>16 mmol $O_2$ / l | 250 µl / min<br>(1.7 VVM) | 50% humidity:<br>6 µl $H_2O$ per day | | |
| | | 200 µl<br>200 µl | 220 rpm<br>300 rpm | 50 mm<br>25 mm | 12 mmol $O_2$ / l<br>12 mmol $O_2$ / l | 250 µl / min<br>(1.3 VVM) | 75% humidity:<br>3 µl $H_2O$ per day | | |

SIMPLE AND LOW COST BIOREACTOR SCALE-DOWN TO MICRO-LITER VOLUMES

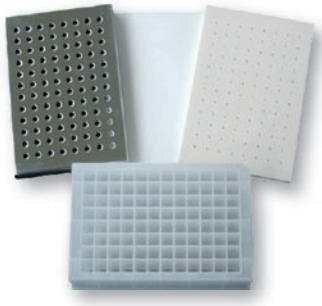
# MicroFlask



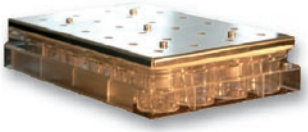
**Sandwich covers for 24-square deep-well plates** allow a culture volume of 2.5 - 4 ml, and are often used for (pellet-forming) streptomycetes and filamentous fungi, e.g. for medium optimization studies or product discovery.



**The cover clamps for deep-well plates** The cover clamp can be mounted on orbital shaking platforms from a range of suppliers.

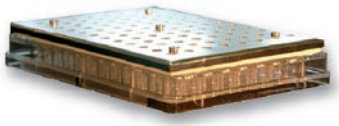


**Sandwich covers for 96-square deep-well plates** allow a culture volume of 0.5 - 1 ml and are most often used for bacterial and yeast cultures.



**Sandwich covers for 24-low-well plates** allow culture volumes of 0.5 - 1 ml and are especially suitable for eukaryotic cell cultures.

**The cover clamps for low-well plates** allow the stacking of up to three 24-well plates or four 96-well plates (hence a maximum of 12 or 16 plates per full clamp). The cover clamp can be mounted on **orbital** shaking platforms from a range of suppliers.



**Sandwich covers for 96-low-well plates** allow a culture volume of 0.1 - 0.2 ml and are most often used for bacterial mutant and construct libraries.

**Literature list** • Duetz, W.A., Rüedi, L., Hermann, R. O'Connor, K, Büchs, J., and Witholt, B. (2000). Methods for intense aeration, growth, storage, and replication of bacterial strains in microtiter plates. Applied and Environmental Microbiology 66:2641-2646. • Minas, W., Bailey, J.E., Duetz, W.A. (2000). Streptomycetes in microcultures: growth, production of secondary metabolites, and storage and retrieval in the 96-well format. Antonie van Leeuwenhoek, 78 (3-4): 297-305 • Duetz, W.A. and Witholt, B. (2001). Effectiveness of orbital shaking for the aeration of suspended bacterial cultures in square deepwell microtiter plates. Biochemical Engineering Journal, 7: 113-115 • Duetz, W.A., Minas, W., Kuhner, M., Clerval, R., Fjallman, A.H.M., Witholt, B. (2001). Miniaturized microbial growth systems in screening. Bioworld 2: 8-10 • Duetz, WA, Witholt, B. (2004). Oxygen transfer by orbital shaking of square vessels and deepwell microtiter plates of various dimensions. Biochemical Engineering Journal 17: 181-185

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