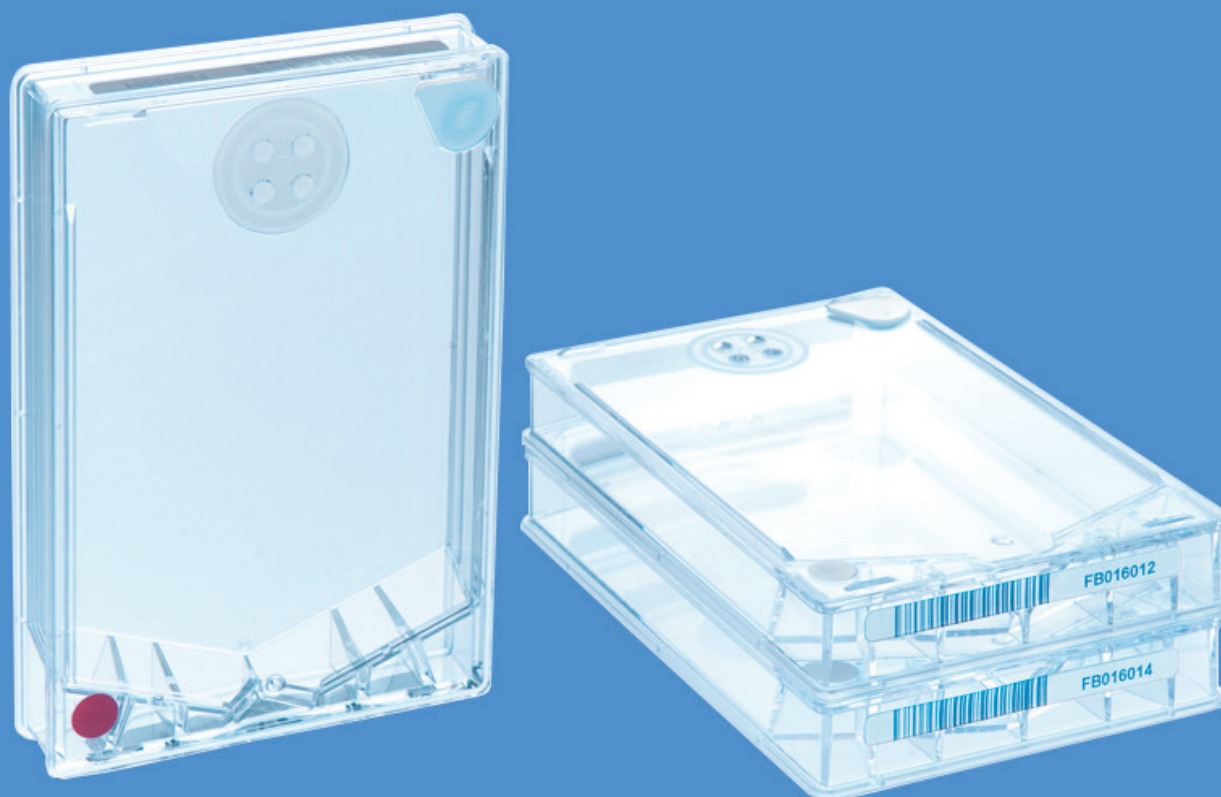


# CELLSTAR® AutoFlask™

Cell culture flask for automated systems

## Instructions for Use



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## 1. INTRODUCTION

The external dimensions of the CELLSTAR® AutoFlask™ correspond to ANSI Standards to render the flask suitable for use on various cell culture, assay and liquid handling systems. The height standard is maintained in order to work with a variety of automation equipment to facilitate manual handling and first steps of automation; e.g. media dispensing and withdrawal. Integrated stacking rims ensure adequate ventilation of each individual flask if stacked in units to maximize use of incubator space.

## 2. CELLSTAR® AUTOFLASK™ PRODUCT PROPERTIES

Features	
• Length	127.76 mm
• Width	85.48 mm
• Height	19.50 mm
• Growth Area	83.60 cm <sup>2</sup>
• Multiple Entry Septum	
• Centrifugation pocket for in flask cell separation	



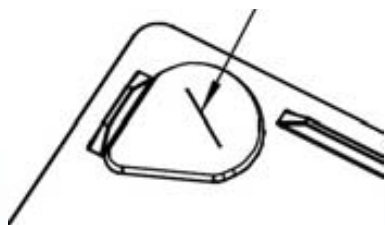
As with all Greiner Bio-One CELLSTAR® products the AutoFlask™ is made of high-grade polystyrene characterized by its exceeding clarity facilitating optical control of cell proliferation and morphology. A proprietary physical surface treatment leading to a hydrophilic surface, significantly increasing the attachment of adherent cells, is provided. Accordingly to the CELLSTAR® product line the CELLSTAR® AutoFlask™ is guaranteed to be sterile, non-pyrogenic and free of RNase and DNase.

## 2.1 Centrifugation pocket

A unique feature of the AutoFlask™ is the centrifugation pocket which enables separation of cells from supernatant inside the flask. Upon horizontal centrifugation cells are collected inside the pocket whereas the medium remains in the bottom vessel of the flask.

(For detailed instruction of use and application see chapter 3 → Automation and manual handling of CELLSTAR® AutoFlask™).

## 2.2 Multiple entry septum



The robot accessible pre-slitted multiple entry septum enables sterile media and solution exchange and the removal of cell based products. Preslitting inhibits coring of the septum by the injection needle, permits reclosing of the septum slit and ensures sterility of the flask content throughout the whole cultivation process. The septum is located at the A1 position to ease programming and piercing with any liquid handling system. Due to the oval shape the slit is oriented diagonally from the

left upper corner to permit tip insertion in this direction. In comparison to competitive products the CELLSTAR® AutoFlask™ has not to be brought in an upright position for flask access. It remains in its horizontal position during the whole cultivation process to minimize any cell stress due to flask moving.

## 2.3 Hydrophobic filter membrane

The integrated filter membrane facilitates gas exchange during the cultivation of cells while providing a sterile barrier against contaminations. The hydrophobic membrane is PTFE coated to prevent wetting of the filter from the internal liquid. Due to the high airflow rate of the filter material and the optimized venting design surpassing oxygen supply is provided to permit cultivation of even very sensitive cells.

## 2.4 Barcoding

The AutoFlask™ is barcoded on both sides with an odd numbering on the septum/filter side and even numbering on the other side. This allows the determination of the flask position by an automated system and efficient automated tracking. The barcode consists of a one-letter-seven-digit barcode starting either with T (TC-treated CELLSTAR® AutoFlask™ version) or S (suspension type flask). If required also customized barcoding can be supplied.

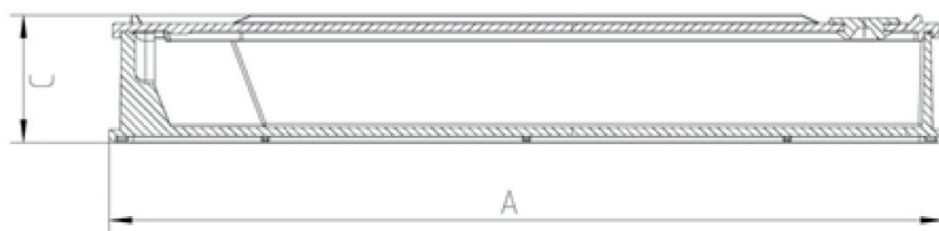
## 2.5 Specific surface treatment

CELLSTAR® AutoFlask™ is provided with a hydrophobic surface for cultivation of suspension cells as well as with a specific tissue culture treatment; significantly increasing the attachment and proliferation of adherent cells. A colored spot on the right corner beneath the centrifugation pocket (P24 position in alignment with a 384 well plate) indicates flask types and facilitates differentiation between suspension version (white dot) and tissue culture treated (red dot).

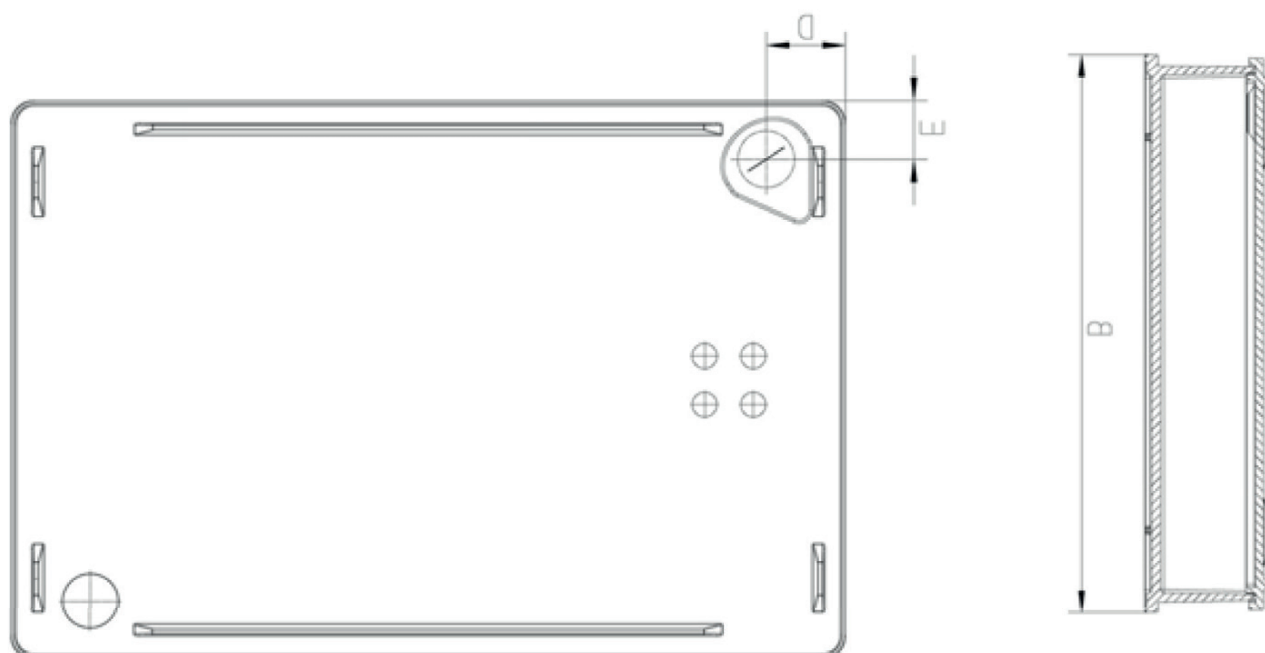
## 2.6 Available product versions

Cat.-No.	Description
779160	AutoFlask™, PS, sterile, TC-treated for adherent cells
779190	AutoFlask™, PS, sterile, for suspension cells

## 2.7 CELLSTAR® AutoFlask™ dimension



	Value [mm]
A	127.76 +/-0.2
B	85.48 +/-0.2
C	19.5 +/-0.25
D	12.3
E	8.99

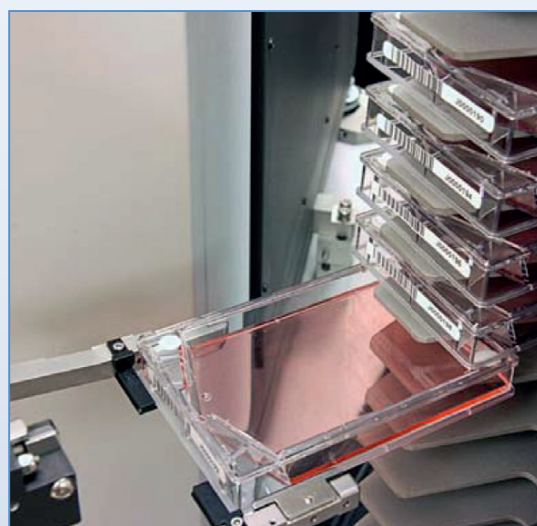


## 3. AUTOMATION AND MANUAL HANDLING OF CELLSTAR® AUTOFLASK™

### 3.1 Automated Handling

As the external dimensions of the CELLSTAR® AutoFlask™ comply with the ANSI Standard the flask is suitable for use on a wide range of cell culture and liquid handling systems. The robotic Automated Compound Profiling System (ACP) from the Genomics Institute of the Novartis Research Foundation (GNF); San Diego (USA) is preprogrammed and validated for AutoFlask™ handling.

Other automated systems using microtiter plate formats may also be programmed for use with CELLSTAR® AutoFlask™. This requests merely the conduction of the injection needle to the A1 position. Contact your equipment supplier for specific details and follow the instruction provided.



## 3.2 Manual Handling

For manual usage of the CELLSTAR® AutoFlask™ the user must apply sterile techniques and ensure a sterile environment such as a laminar flow. Exchange of liquids should be performed through the septum to minimize any risk of contamination. Removal of the septum is not recommended. Due to the exceeding slit size disposable tips as well as blunt end metal cannulas with a maximum diameter of 2,5 mm can be inserted through the septum. If a liquid handling system is available a semiautomated usage of the CELLSTAR® AutoFlask™ can be realized facilitating filling and removal of media through this system and therefore improving sterility conditions of cultivation. Please contact your equipment supplier for further details to facilitate single pipetting at the A1 position. Stacking rims on the upper side enable stable piling of various flasks inside the incubator creating an effectual interspace between the lower and the upper flask to ensure sufficient ventilation of each individual flask through the hydrophobic filter membrane. This interspace also averts contamination of the outer septum surface by the upper flask bottom to assure sterility of flask content by septum piercing. To separate cells from supernatant a specific centrifugation adapter is provided by Greiner Bio-One compatible with Hettich Rotaria centrifuges with a swinging bucket rotor. Depending on media content a maximum centrifugation force between 2500 rpm (30 ml of media) and 1500 rpm (60 ml) can be applied for cell and media separation whereas the recommended centrifugation force for cell collection refers to 800–1000 rpm.

## 3.3 Protocol for cell harvest of adherent cells

1. Withdraw cultivation media through the septum
2. Add 5-10 ml of PBS or non-serum-containing media and draw off as described above
3. Fill in 3 ml of Trypsin/EDTA solution and incubate at 37 °C and 5% CO<sub>2</sub> for 5-10 minutes
4. Add the equivalent amount (3 ml) of serum containing media to stop enzyme digestion
5. Remove cell suspension through the septum and transfer to the adequate amount of new flasks

## 3.4 Protocol for cell harvest of suspension cells

1. Centrifuge CELLSTAR® AutoFlask™ with 800-1000 rpm using the appropriate centrifugation adapter to accumulate cells inside the centrifugation pocket.
2. Withdraw cultivation media through the septum
3. Add 5 ml of serum-containing media and softly move the flask to resuspend collected cells
4. Remove cell suspension through the septum and transfer to the adequate amount of new flasks

## 4. ADVANTAGES OF CELLSTAR® AUTOFLASK™

In the horizontal position, the flask can be manipulated like any ordinary microplate, with numerous existing automation options. The injecting needle can be easily programmed and conducted to the A1 position as no vertical position is needed to gain flask access. Due to the maximal slit size disposable tips as well as blunt end metal cannulas with a maximum diameter of 2,5 mm can be inserted through the septum. In comparison to a T-75 Flask the CELLSTAR® AutoFlask™ reaches in a high throughput compatible format a growth area of 83.6 cm<sup>2</sup>, occupying less than half of the incubator space. Due to the high airflow rate of the filter material and the optimized venting design surpassing oxygen supply is provided to permit cultivation of even very sensitive cells. The unique centrifugation pocket enables the separation of cells and supernatant inside the flask maintaining a closed sterile environment during this process.

## 5. CELLSTAR® AUTOFLASK™ ORDERING INFORMATION

Cat.-No.	Description	Qty/ bag	Qty/ case
779160	AutoFlask™, PS, sterile, TC-treated for adherent cells	10	100
779190	AutoFlask™, PS, sterile, for suspension cells	10	100