

CARLSBERG FLASK

USER MANUAL



THIS IS THE FIRST VERSION OF THE CARLSBERG FLASK MANUAL

The table below lists previous versions of this User Manual and states the major changes between versions.

Version #	Version date	Major changes from previous versions
1	December 2016	This is the first version of the Carlsberg Flask manual

INTRODUCTION:

MANUFACTURER: Keofitt A/S
Kullinggade 31
5700 Svendborg, Denmark

TYPE: Carlsberg Flask (item No. 200001)
CERTIFICATES:

- Regulation (EC) No. 1935/2004
- PED 2014/68/EU of the European Community, Category I
- PED 2014/68/EU of the European Community, Fluida Group II

YEAR OF INTRODUCTION: 2016
MANUAL LAST UPDATED: December 2016

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IMPORTANT INFORMATION

EC DECLARATION OF CONFORMITY

The company: Keofitt A/S
Kullinggade 31
DK-5700 Svendborg

represented by the undersigned hereby declare that the product:

Carlsberg Flask (item No. 200001)

is in conformity with the following directives with amendments:

- Regulation (EC) No. 1935/2004
- PED 2014/68/EU of the European Community, Category I
- PED 2014/68/EU of the European Community, Fluida Group II

Svendborg, 12th December 2016

KEOFITT®
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Henrik L. Salomon, Managing director

Always read this manual carefully before using the Carlsberg Flask

This instruction manual is part of the delivery.

Since a Keofitt W9 valve and a Keofitt MicroPort are part of the Carlsberg Flask the respective manual are also included in the delivery.

Study the instructions carefully.



IMPORTANT

Keofitt A/S cannot be held responsible for incorrect assembly and use of the Keofitt Carlsberg Flask.

1. PRESENTATION

The Carlsberg Flask (hereafter designated “CF” or “flask”) is a 30-litre vessel system designed for the propagation of yeast cultures in a sterile environment and the transfer of the pure yeast culture under sterile conditions ensuring contamination-free culture development and transfer.

It is primarily used in breweries, but may also be used for other similar tasks within yogurt production, biotech or the pharmaceutical industry.

The CF system is designed to provide a method to grow cultures under ideal conditions in a sterile environment minimizing or eliminating unwanted agents from acting on the culture growth. The system is designed of stainless steel and food grade materials, with valving, instrumentation and components to effectively grow the cultures and transfer them in a sterile condition.

The term “flask” is used to designate the entire system, whereas the term “vessel” only refers to the container or the tank.



INFORMATION

There are many slight nuances that CF users utilize based on experience and peripheral equipment available, and it is Keofitt’s intention to outline a method for use of the CF, but not the only method. It is imperative to operate the equipment in a safe manner with safe operating practices by knowledgeable users using the necessary safety equipment and procedures.



WARNING

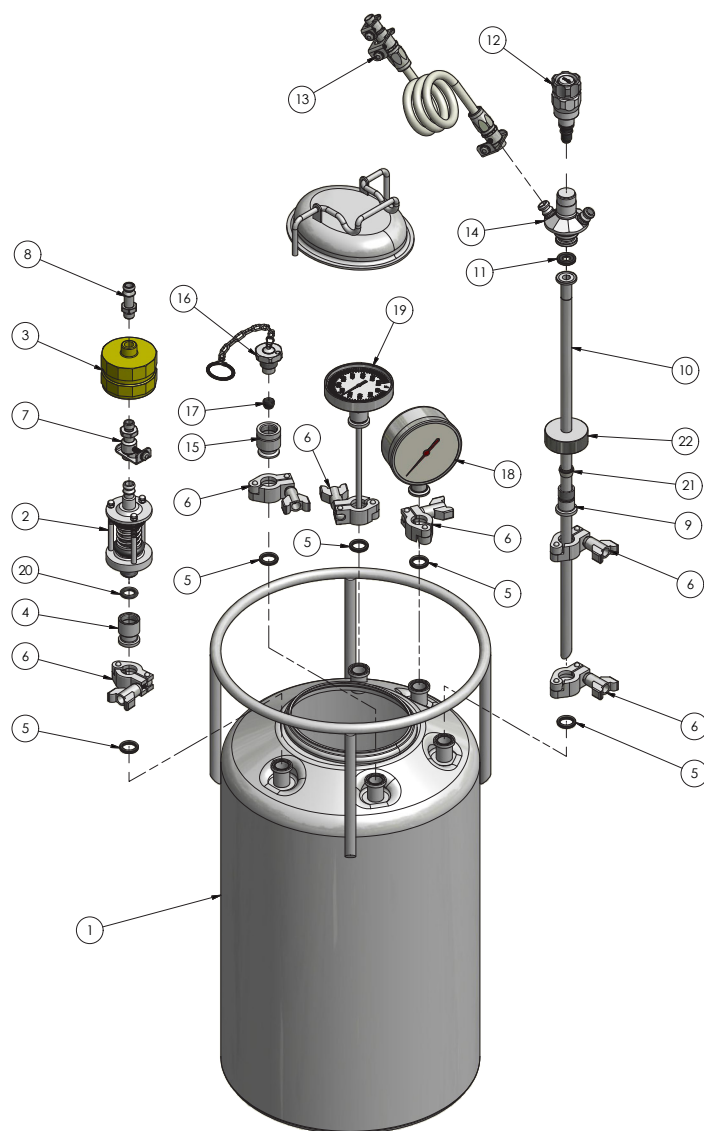
The operating instructions are consequently general in nature (although often relating to breweries) and must not be considered as a guideline to any specific process in terms of quantities, times, temperature, pressure etc. The user should rely on their own experience and the knowledge and experience of their yeast supplier and experience with their own cultures and standard operating procedures.

1.1 Description

The CF consists of the following main components:

#	Name (drawing reference)	Description
1	Vessel (1)	Stainless steel flat-bottomed container with a welded ring-shaped handle forming a cage to protect the instrumentation and connections
2	Temperature gauge (19)	Thermometer measuring the temperature in the middle of the vessel. Available in either °C or °F.
3	Manometer/pressure gauge (18)	Manometer indicating the pressure inside the vessel. Available in either psi or bar.
4	MicroPort septum (15/16/17)	Allows inoculation of liquid additives, like yeast culture. Using a long needle it is also possible to extract a sample.

5	Bung valve (2)	This valve is a combined relief valve AND check valve (non-return valve). It acts as a relief valve against too high pressure in the vessel. The pressure setting is adjustable to 0-2 bar(g). It also acts as a check valve allowing pressurised air from outside to enter the vessel.
6	Sterile filter (3)	The sterile filter is placed on top of the bung valve and filters the pressurised air before it enters the vessel. The sterile filter is foreseen with a Keofitt Quick Coupling for easy connection to the bung valve and a Keofitt hose piece for easy connection to a pressurised sterile air hose.
7	Transfer valve (12/14)	The transfer valve is fitted on a long piece of steel tubing (ascension pipe or dip pipe), which height can be adjusted in order to obtain different heights of the pipe end inside the vessel. The transfer valve is identical to a Keofitt W9 sampling valve.
8	Lid (1A/1B)	The vessel is completely sealed off and may be pressurised, when the lid is in place.
9	Transfer hose (13)	A flexible hose made from PTFE, provided with Keofitt stainless steel Quick Couplings. Available as standard 0.5 m and 1.0 m lengths, with custom lengths available.



PARTS LIST			
ITEM	QTY	PART No	DESCRIPTION
1	1	CBF-25-10	
2	1	900039	Relief Valve
3	1	200211	
4	1	CBF-00-15	Adaptor to 3/4" Tri-Clamp
5	5	4509K12	Tri-Clamp Gasket, 3/4"
6	6	45195K44	Medium Pressure Clamp, Wing Nut, for 3/4" & 1/2" OD
7	1	900130	
8	1	900031	
9	1	CBF-00-10	
10	1	CBF-00-20	
11	1	4509K11	Tri-Clamp Gasket, 1/2"
12	1	Part8	
13	1	550003	
14	1	850036	
15	1	CBF-00-05	Tri-Clamp Connecton
16	1	900056	
17	1	900049	
18	1	EL031010041011A	EL-Standard 90mm Sanitary Pressure Gauge
19	1	SGT300R	Thermometer, 3" Dial
20	1	900829	O-Ring
21	1	T-810-SET	
22	1	CBF-00-11	

2. CARLSBERG FLASK FUNCTION

The CF is a vessel system that is used to propagate cultures to increase the number for use in a fermentation process. As the primary or near primary growth vessel it is imperative to eliminate unwanted growth factors early to ensure the rigorous growth of the desired cultures.

The CF components ensure a sterile vessel, with multiple ways to sterilize based on the heating source available to the user. Transfer of the cultures is handled through a series of transfer valves – one provided as part of the CF and a second one located at the recipient tank. This allows a completely sterilized transfer path to maintain system sterility and the means to transfer liquids (and some suspensions) in and out in a sterile fashion.

The CF allows the user to obtain a sterile environment inside the vessel and to add liquid substances (additives) without compromising the sterility. Dry yeast is normally re-hydrated for $\frac{1}{2}$ - 1 hour to allow the yeast to take in water and rebuild the cell membrane/structure before introducing the culture into the CF system.

The vessel can be sterilised in a number of ways: by heating water prior to introduction of the base medium or base medium can be sterilised directly in the flask from the start.

All the related tank components must be cleaned thoroughly before assembly and will be sterilised in-situ as the flask content is brought to a boil and kept there for a sufficient amount of time to obtain sterility.

The CF is flat-bottomed allowing heating by means of a heating plate. Other means of heating include the use of a gas burner or autoclaving, the latter requiring a very large autoclave to accommodate the flask. The CF can be autoclaved with the flask components in tact. (Please notify Keofitt of the specific means of sterilizing to ensure it meets your requirements). In general, the flask can be provided with components to accommodate autoclaving at 121 C for 15 minutes.

3. CARLSBERG FLASK INSTALLATION

3.1 Unpacking


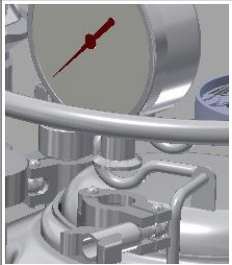

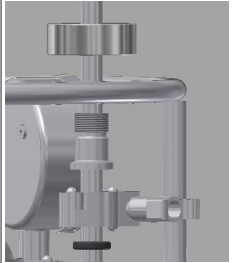

Gauges, valve and similar components are delivered either attached or detached. Make sure all parts are supplied according to the parts list above. Check all parts for visible transport damage.


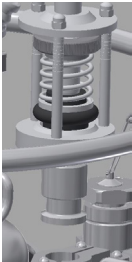
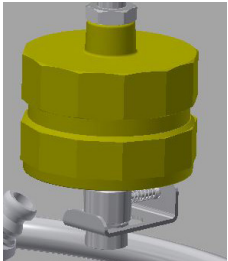
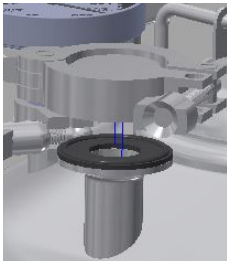
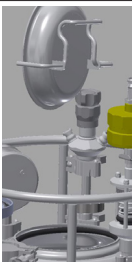
3.2 Cleaning

Components already fitted on delivery must be detached for cleaning. Clean all parts thoroughly before final assembly using an appropriate detergent. Fit the rubber caps on the transfer valve after cleaning.

3.3 Assembly

The various parts must be assembled as follows:

1.	Fit the thermometer using a Mini Tri-clamp and corresponding gasket	
2	Fit the manometer using a Mini Tri-clamp and corresponding gasket	
3	Fit the MicroPort using a Mini Tri-clamp and corresponding gasket	
4.	Fit the dip pipe assembly using a Mini Tri-clamp and corresponding gasket	
5.	Fit the W9 valve using a Mini Tri-clamp and corresponding gasket	

6.	Adjust the height of the dip pipe by first loosening the knurled ring, then adjusting the height and finally tightening the knurled ring again. Typically the dip tube is completely inserted.	
7.	Fit the Bung valve on to the vessel using a Mini tri-clamp and corresponding gasket. The bung valve is adjustable and should be set at the lowest setting. See the separate bung valve operation instruction.	
8.	Fit the filter housing on to the bung valve	
9.	Fit the blank disk and diffuser using a 1.5" tri-clamp and corresponding gasket	
10.	The lid is fitted by lowering it vertically into the vessel while holding it by the handle – After turning the lid in the horizontal position pull it up against the vessel opening and lock it by lowering the handle flat down towards the lid. The locking mechanism should “snap” into place.	

4. CARLSBERG FLASK OPERATIONS

4.1 Cleaning

Components already fitted on delivery must be detached for cleaning.

Clean all parts thoroughly before final assembly using an appropriate detergent.

Fit the rubber caps on the transfer valve after cleaning.

4.2 Sterilisation

There are two conditions for sterilizing the CF:

- 1.** Having the vessel filled with water
- 2.** Having the vessel filled directly with the process medium, like wort for beer brewing

In either case the sterilisation takes place in an autoclave, on a gas burner or on an electric heating plate, but in solution 1 the water has to be expelled from the vessel by pressurised sterile air and an already sterile process medium transferred aseptically into the vessel. In solution 2 the process medium is already in the vessel and will be sterilised together with the vessel.

The following procedure for solution 1 (water) is recommended for the sterilisation using a gas burner or heating plate:

- 1.** Fill the flask to its net volume (approx. 25% of total volume) with water
- 2.** Close the lid
- 3.** Make sure all gauges and other attachments are properly mounted and working as expected
- 4.** Place the flask on the heat source and boil the water for 15-20 minutes
- 5.** Remove one rubber cap and connect a hose to the transfer valve and place the other end of the hose in a position lower than the CF
- 6.** Open the transfer valve slowly and the pressure, which has built up in the vessel will force most of the water out
- 7.** Connect pressurised sterile air to the sterile filter to force any remaining water out of the flask
- 8.** Close the transfer valve after the water has been expelled
- 9.** Disconnect the sterile air supply and the hose
- 10.** Put the CF in cold water or in a refrigerator to cool it down
- 11.** After cooling the CF is sterile and ready to receive the base substance (e.g. the wort) by transfer as explained in the transfer valve operating instructions

The following procedure for solution 2 (wort or other process medium) is recommended for the sterilization using a gas burner or heating plate:

- 1.** Fill the flask to its net volume (approx. 75-80% of total volume) with wort or other nutrient rich medium satisfactory for cell growth.
- 2.** Close the lid
- 3.** Make sure all gauges and other attachments are properly mounted and working as expected
- 4.** Place the flask on the heat source and bring the temperature to a boil for 15-20 minutes. It is only necessary to heat the wort/wort substitute to pasteurization temperature of approximately 66 C for 15 minutes. But when using wort as a vessel sterilant, bring wort to a boil to sterilize the vessel internal environment, before cooling.
- 5.** Put the CF in cold water or in a refrigerator to cool it down
- 6.** After cooling the CF is sterile and ready for the inoculation as explained in the transfer valve operation or Microport transfer operating instructions.



WARNING

- Be careful as steam will occasionally flow out of the bung valve (relief valve)

4.3 Aeration

Aeration of the wort is carried out by forcing sterile air through the transfer valve, causing it to bubble through the wort. As pressure increases the air will eventually escape through the bung valve and a flow of air is obtained. The flow should be sufficiently slow to avoid excess foaming and minimize pressure in the vessel, as this may affect the fermentation.

The procedure for aeration is as follows:

- 1.** Make sure the transfer valve is closed
- 2.** Connect a steam hose to one of the connection pieces
- 3.** Connect a hose to the other connection piece and let it go to drain
- 4.** Sterilise the valve for 60 sec. by steaming
- 5.** Disconnect the exhaust hose and fit an autoclave-treated rubber cap (or steel cap) to the connection piece
- 6.** Disconnect the steam hose and immediately connect the sterile air hose to the connection piece using an autoclave-treated Quick coupling fitted to your sterile air hose
- 7.** Set the pressure relief valve to the desired pressure (0-1 bar(g))
- 8.** Open the transfer valve to enable sterile air to flow into the Carlsberg Flask
- 9.** Close the valve after aeration is complete, but continuous slow aeration will aid the fermentation.
- 10.** Disconnect the sterile air supply and mount the protective rubber cap

4.4 Innoculation

When the process is ready yeast or any other liquid substance can be aseptically added to the process in one of two ways:

1. Through the MicroPort septum
2. Through the transfer valve

For smaller quantities of low-viscous liquids (with no solid particles) **MicroPort Operation** is the easiest and fastest. Proceed as follows:

1. Prepare your syringe with the liquid to inject, syringe of about 1 mm inner diameter.
2. Disinfect the needle on the syringe with alcohol or another appropriate disinfectant
3. Remove the plug from the MicroPort
4. Remove (unscrew) the cotton wick (if present) and discard it
5. Clean and disinfect the septum using a new wick soaked in an appropriate disinfectant
6. Discard the wick
7. Immediately after cleaning/disinfecting pierce the septum with the syringe holding it at a slight angle relative to the axes of the Micro Port;
8. Empty the syringe into the vessel and pull the syringe out
9. If an additional hygiene barrier is required, place (screw in) a new wick in the plug soaked in alcohol or another appropriate disinfectant. Put the plug back in place after having lubricated the o-ring with an appropriate lubricant for easy removal in subsequent uses.

Alternatively, make use of the Transfer Valve Operation to transfer the additive from a sterile container into the flask. To assure aseptic transfer the container holding the additive must be fitted with a steam sterilizable valve, like a Keofitt W9 sampling valve.

In this case, please proceed as follows:

1. Connect the steam supply to one connection piece of the valve on the additive container
2. Connect a Teflon hose from the other connection piece of the above-mentioned valve to a connection piece of the CF's transfer valve
3. Connect another Teflon hose to the other connection piece of the transfer valve and let it go to drain
4. Sterilise the flow path by applying steam for 1-2 minutes
5. Disconnect the steam and fit a sterile (autoclaved) rubber cap on the connection piece
6. Disconnect the drain hose and fit a sterile (autoclaved) rubber cap on the connection piece
7. Make sure the pressure in the additive container is higher than in the CF to assure flow
8. Open the additive container valve first, then the transfer valve to enable transfer of liquid from the from the additive container to the CF

- 9.** Once the transfer is completed (bubbling noise in the vessel), allow a few more seconds to make sure all the additive has been transferred, then close the valves (transfer valve first)
- 10.** Reconnect the steam hose and drain hose
- 11.** Rinse the flow path using steam or some other appropriate means
- 12.** Disconnect steam hose and drain hose

4.5 Sampling

Samples may be taken in one of two ways:

- 1.** A needle through the MicroPort
- 2.** By means of the transfer valve

When adopting solution 1 make sure to use a needle, which is sufficiently long to reach the fluid surface.

When adopting solution 2 proceed as described in separate transfer procedure, but only transfer a small sample.

4.6 Transfer

The transfer of the content from the CF to another tank (the receiving vessel) must be carried out under aseptic conditions. Therefore the flow path (the transfer path) consisting of 2 valves and a hose must be steam sterilised before transfer takes place. If steam is not available, alcohol or another appropriate disinfectant may be used.

Unless the receiving vessel is lower than the CF (and liquid can flow by gravity), sterile air pressure to the CF is required in order to press the liquid from the CF to the tank.

To prepare for the transfer and to carry it out, please proceed as follows (assuming a Keofitt valve on the tank):

- 1.** Connect the steam supply to one connection piece of the transfer valve on the CF
- 2.** Connect a Teflon hose from the other connection piece of the transfer valve to a connection piece of the tank valve
- 3.** Connect an other Teflon hose to the other connection piece of the tank valve and let it go to drain
- 4.** Sterilise the flow path by applying steam for 1-2 minutes
- 5.** Disconnect the steam and fit a sterile (autoclaved) rubber cap on the connection piece
- 6.** Disconnect the drain hose and fit a sterile (autoclaved) rubber cap on the connection piece
- 7.** Supply compressed sterile air to the bung valve in order to provide sufficient pressure to force the liquid into the tank
- 8.** Open the CF transfer valve first, then the tank valve to enable transfer of liquid from the CF
- 9.** Once the transfer is completed (bubbling noise in the tank), allow a few more minutes to make sure all liquid has been transferred, then close the valves (tank valve first)
- 10.** Disconnect compressed sterile air supply

- 11.** Reconnect the steam hose and drain hose
- 12.** Rinse the flow path using steam or some other appropriate means
- 13.** Disconnect steam hose and drain hose
- 14.** Disassemble CF and clean all parts ready for next use

5. MAINTENANCE

Apart from regular cleaning the following maintenance activities are required at regular intervals:

- 1.** Replace the valve membrane (see W9 User Manual)
- 2.** Replace the MicroPort septum after each production batch (see MicroPort User Manual)
- 3.** Inspect the lid's O-ring and replace if any signs of wear



WARNING

- Never perform any service activities on the CF when it is HOT
- Never perform any service activities on the CF when UNDER PRESSURE. Under normal operating conditions, the CF should not exceed more than a low pressure condition.

6. TECHNICAL DATA

For further product information - material, dimensions etc. - please refer to the specific datasheet at www.keofitt.dk

The current data sheet is shown below.

CARLSBERG FLASK

ART. NO. 200001

GENERAL

The Carlsberg Flask is used for the propagation of yeast cultures in a sterile environment and the transfer of the pure yeast culture under sterile conditions ensuring contamination-free culture development.

FLASK FEATURES

- Sterilization of flask environment and fermentation media by hot plate, external flame, or autoclave
- Dual-action tank inlet valve for vessel protection and sterile air supply without loss of pressure or exposure to atmospheric conditions
- Inoculation /extraction septum valve
- Patented sterile transfer valve and sterile hose assemblies
- Vessel pressure gauge
- Vessel temperature gauge

FLASK BENEFITS

- Effective environment sterilization for contamination-free propagation
- Method of inoculation, culture transfer and vessel protection never compromises culture sterility
- Flask environment conditions can be monitored can be monitored for ideal fermentation conditions
- Patented transfer valve with transfer system

TECHNICAL SPECIFICATION

- Approx. 30 liter standard gross sizes – other sizes available on request
- 316L material of construction
- Electro-polished finish 5-10 RA
- Dimensions: 30 liter - 660 mm (height) - Ø300 mm
- Comes complete with vessel, pressure relief valve / Sterile air injection valve, sterile filter housing, sterile filter cartridges, inoculation septum, pressure gauge, temperature gauge, sterile transfer valve, 3 ft transfer line with stainless couplings

FLASK METHOD

- The flask can be sterilized with water prior to introduction of the base media or base media can be sterilized in the flask from the start.
- All the related tank components can be sterilized in-situ as the flask contents are brought to a boil for sterilization.
- Pressure and cool down temps can be monitored.
- Inoculation is done through the septum valve with syringe, through the transfer valve via needle-less syringe, or sterile transfer bag.
- Transfers are done in a pre-sterilized transfer path.



7. PARTS LIST

For spare parts and general inquiries please make reference to the item numbers and descriptions below.

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PARTS LIST			
ITEM	QTY	PART No	DESCRIPTION
1	1	CBF-25-10	
2	1	900039	Relief Valve
3	1	200211	
4	1	CBF-00-15	Adaptor to 3/4"Tri-Clamp
5	5	4509K12	Tri-Clamp Gasket, 3/4"
6	6	4519SK44	Medium Pressure Clamp, Wing Nut, for 3/4" & 1/2" OD
7	1	900130	
8	1	900031	
9	1	CBF-00-10	
10	1	CBF-00-20	
11	1	4509K11	Tri-Clamp Gasket, 1/2"
12	1	Part8	
13	1	550003	
14	1	850036	
15	1	CBF-00-05	Tri-Clamp Connecton
16	1	900056	
17	1	900049	
18	1	EL031010041011A	EL-Standard 90mm Sanitary Pressure Gauge
19	1	SGT300R	Thermometer, 3" Dial
20	1	900829	O-Ring
21	1	T-810-SET	
22	1	CBF-00-11	

Keofitt reserves the right to change technical data without notice!

For complete set of updated data sheets and manuals for Keofitt products please refer to our web page www.keofitt.dk



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