AUTOCLAVABLE BIOREACTOR 2 – 7 LITER

USER MANUAL

V3UBCE0061

Document Version 1.5

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1 SAFETY

1.1 SYMBOLS

The following symbols are used on the equipment and in this manual.

WARNING
Important issue. Refer to this manual.

Additional information

1.2 SAFETY WARNINGS

GENERAL

- The Hardware manual contains information and warnings, which have to be followed by the user to ensure safe installation, operation and to retain the equipment in safe condition. Carefully read this manual before putting the autoclavable bioreactor into operation!
- This autoclavable bioreactor is designed for biotechnological experiments; it must not be used for other purposes!
- The autoclavable bioreactor is mostly used as a Stirred Tank Reactor. This means that after autoclaving the bioreactor, the Stirrer Motor will be mounted on top of the Stirrer Assembly. Do not operated the Stirrer Motor until it is properly seated in the Stirrer Assembly. For safety instructions regarding mounting and operation of the Stirrer Motor, refer to the Instrument / User Manuals of the corresponding stirrer motor control device.

WARNING
Risk of overpressure in the glass bioreactor.

- The glass bioreactor is damaged easily (scratches on the surface)! As a result, its overall strength is reduced. Therefore, do not apply a process pressure that exceeds 0.5 barg (7 psig). Reduce the pressure of the inlet gasses to this extend or apply a pressure relief valve in the top plate of the reactor that is adjusted accordingly.

WARNING:
Gas leakage due to O-ring wear.

- Auxiliary connections at the reactor head plate are sealed by silicone O-rings and bearings. Due to wear of these seals, the assembled bioreactor will become less gastight. Replace the O-rings and bearings on a regular basis (e.g. once a year).

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2 HARDWARE SPECIFICATION

2.1 GENERAL

The stirred tank reactors (STR) are the most applied reactor types in biotechnology.
The Applikon 2 - 7 liter autoclavable bioreactors offer several advantages; its modular and flexible design requires minimum bench space and is very practical in operation.

In combination with Applikons stirrer assemblies, motors, impellers and auxiliaries, you can configure the reactor for a variety of applications, such as:
- medium optimization,
- screening of strains,
- product optimization,
- scale up/down studies,
- reactor optimization,
- continuous cultures,
- perfusion systems, etc.

2.2 CLEAN BEFORE FIRST USE

This manual describes a standard (non-autoclave) cleaning procedure for glass bioreactors. A more extensive cleaning procedure used only for cleaning glass bioreactors before their first use runs as follows:

1. Remove sensors and other auxiliaries (clean these separately)
2. Fill the reactor with 0.2M NaOH at 50°C
3. Activate the stirrer at high speed and run for 1 hour
4. Rinse twice with demi water
5. Fill the reactor with 1% citric acid and 0.1% EDTA at 50°C
6. Activate the stirrer at high speed and run for 1 hour
7. Rinse twice with demi water and dry with clean air

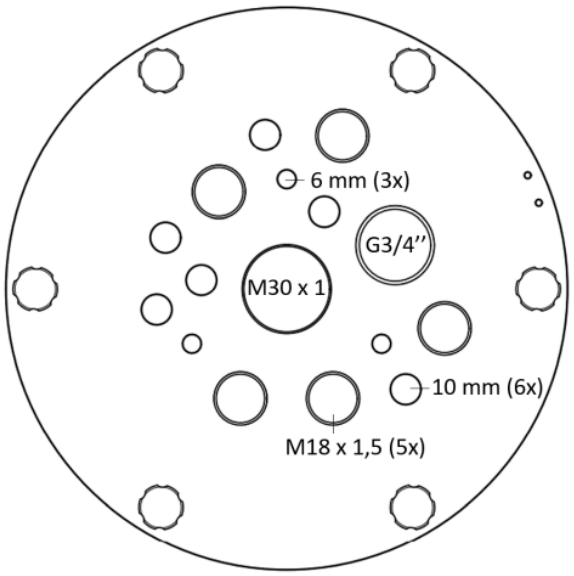
2.3 THE REACTORS

The 2 - 7 Liter bioreactors are ideal as tool for researchers who start fermentation studies and for those applications where small or medium volumes are required.
Due to the modular design, the reactors can be used for a variety of applications, including microbial and yeast fermentations, cell cultures, etc.

Reactor material:	borosilicate glass
Other materials in contact with the medium:	stainless steel (head plate) silicon rubber viton and EPDM (optional)

The reactor types and their specifications are listed in the sections below.

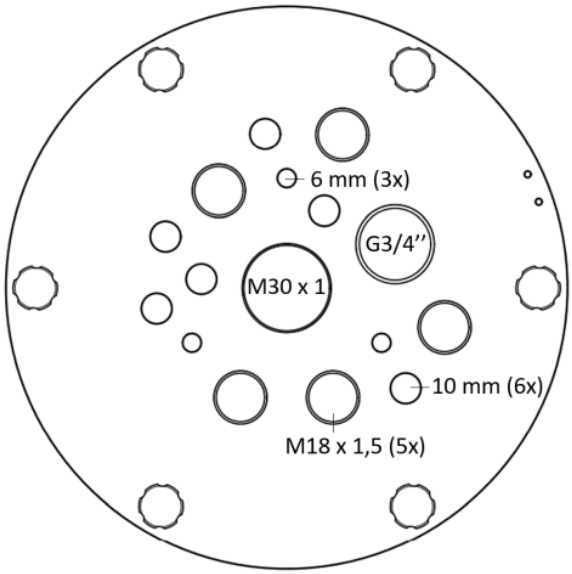
2.3.1 2 LITER REACTOR



Reactor Type	2 liter, dished bottom
Inner Diameter	105 mm
Inner Height (maximum)	240 mm
Liquid Height (working volume)	156 mm
Required Autoclave Space	400 x 200 mm
Req. Autocl. Space with Condenser	460 x 200 mm
Overall Height Reactor	290 mm
Total Volume	2.2 liter
Working Volume	1.7 liter
Minimum Working Volume	0.3 liter
H/D Total	2.3
H/D Working Volume	1.9
Volume Indication	Present
Minimum Reactor Pressure	-1 barg
Maximum Reactor Pressure	0.5 barg
Ports in Head Plate	1 * M30 x 1
	1 * G3/4"
	5 * M18 x 1.5
	3 * 6 mm, 6 * 10 mm

The head plate also contains two 2 mm holes for level sensor connection.

Z611000210 2-liter Dished-bottom Reactor

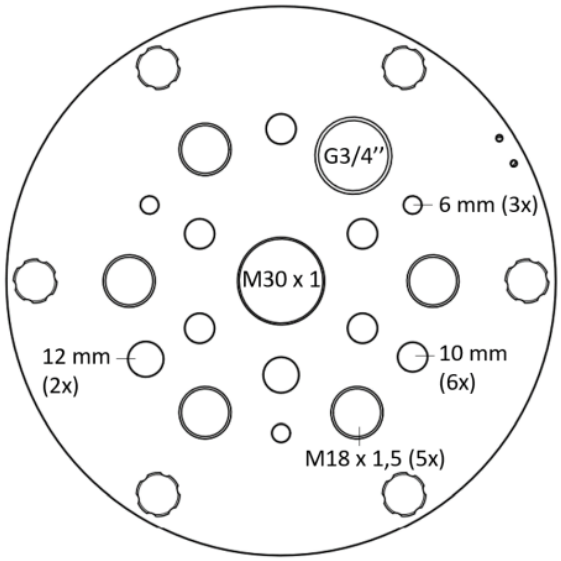


Reactor Type	2 liter, jacketed
Inner Diameter	105 mm
Inner Height (maximum)	240 mm
Liquid Height (working volume)	156 mm
Required Autoclave Space	400 x 240 mm
Req. Autocl. Space with Condenser	460 x 240 mm
Overall Height Reactor	290 mm
Total Volume	2.2 liter
Working Volume	1.7 liter
Minimum Working Volume	0.3 liter
H/D Total	2.3
H/D Working Volume	1.9
Jacket Volume	1.4 liter
Volume Indication	Present
Minimum Reactor Pressure	-1 barg
Maximum Reactor Pressure	0.5 barg
Ports in Head Plate	1 * M30 x 1
	1 * G3/4"
	5 * M18 x 1.5
	3 * 6 mm, 6 * 10 mm

The head plate also contains two 2 mm holes for level sensor connection.

Z611000220 2-liter Jacketed Reactor

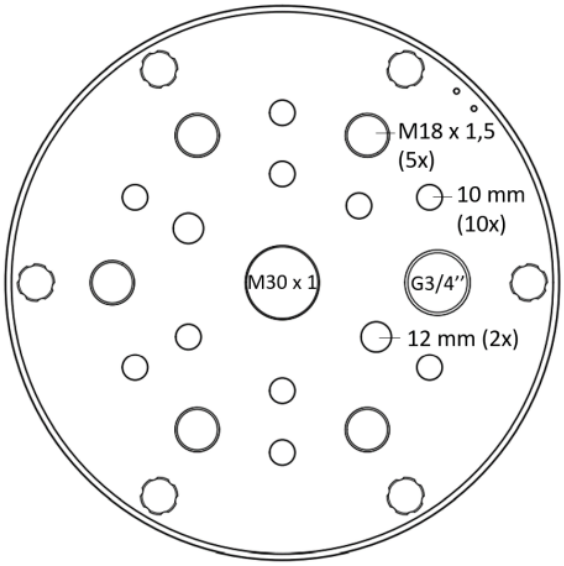
2.3.2 3 LITER REACTOR



Reactor Type	3 liter, dished bottom
Inner Diameter	130 mm
Inner Height (maximum)	250 mm
Liquid Height (working volume)	200 mm
Required Autoclave Space	400 x 200 mm
Req. Autocl. Space with Condenser	460 x 200 mm
Overall Height Reactor	290 mm
Total Volume	3.1 liter
Working Volume	2.4 liter
Minimum Working Volume	0.6 liter
H/D Total	1.9
H/D Working Volume	1.5
Volume Indication	Present
Minimum Reactor Pressure	-1 barg
Maximum Reactor Pressure	0.5 barg
Ports in Head Plate	1 * M30 x 1
	1 * G3/4"
	5 * M18 x 1.5
	3 * 6 mm, 6 * 10 mm, 2 * 12 mm

The head plate also contains two 2 mm holes for level sensor connection.

Z611000310 3-liter Dished-bottom Reactor

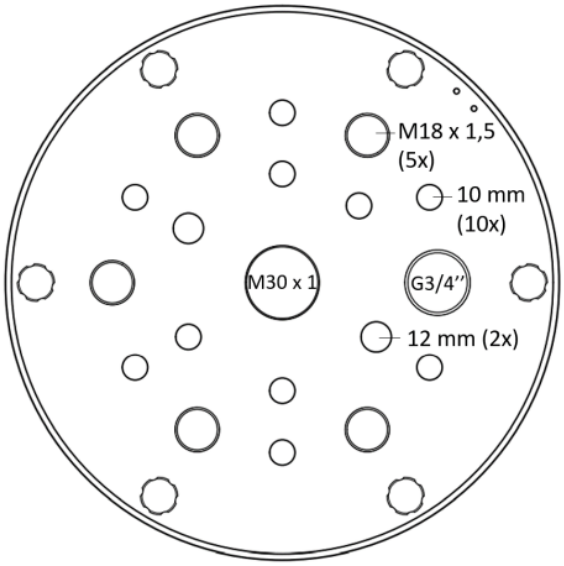


Reactor Type	3 liter, jacketed
Inner Diameter	130 mm
Inner Height (maximum)	250 mm
Liquid Height (working volume)	200 mm
Required Autoclave Space	400 x 240 mm
Req. Autocl. Space with Condenser	460 x 240 mm
Overall Height Reactor	290 mm
Total Volume	3.1 liter
Working Volume	2.4 liter
Minimum Working Volume	0.6 liter
H/D Total	1.9
H/D Working Volume	1.5
Jacket Volume	1.2 liter
Volume Indication	Present
Minimum Reactor Pressure	-1 barg
Maximum Reactor Pressure	0.5 barg
Ports in Head Plate	1 * M30 x 1
	1 * G3/4"
	5 * M18 x 1.5
	3 * 6 mm, 6 * 10 mm, 2 * 12 mm

The head plate also contains two 2 mm holes for level sensor connection.

Z611000320 3-liter Jacketed Reactor

2.3.3 5 LITER REACTOR

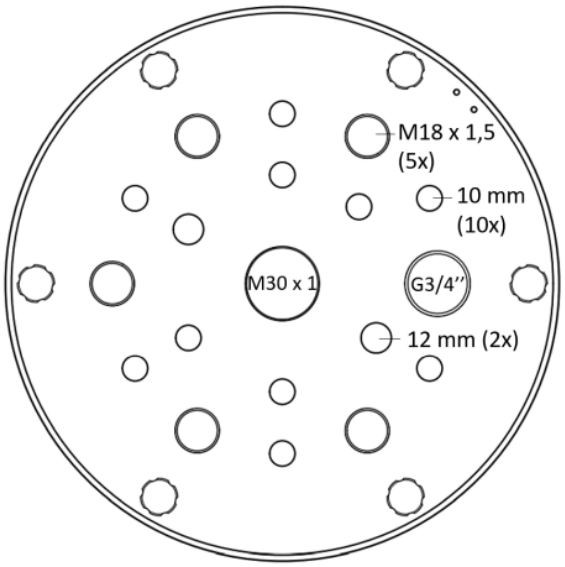


Reactor Type	5 liter, dished bottom
Inner Diameter	160 mm
Inner Height (maximum)	250 mm
Liquid Height (working volume)	180 mm
Required Autoclave Space	400 x 200 mm
eq. Autocl. Space with Condenser*	520 x 200 mm*
Overall Height Reactor	290 mm
Total Volume	4.8 liter
Working Volume	3.4 liter
Minimum Working Volume	0.9 liter
H/D Total	1.6
H/D Working Volume	1.1
Volume Indication	Present
Minimum Reactor Pressure	-1 barg
Maximum Reactor Pressure	0.5 barg
Ports in Head Plate	1 * M30 x 1
	1 * G3/4"
	5 * M18 x 1.5
	10 * 10 mm, 2 * 12 mm

*The given dimensions for the required autoclave space are valid for the condenser for microbial applications. The condenser that can be used for cell cultures is 60 mm smaller (less tall).

The head plate also contains two 2 mm holes for level sensor connection.

Z611000510 5-liter Dished-bottom Reactor



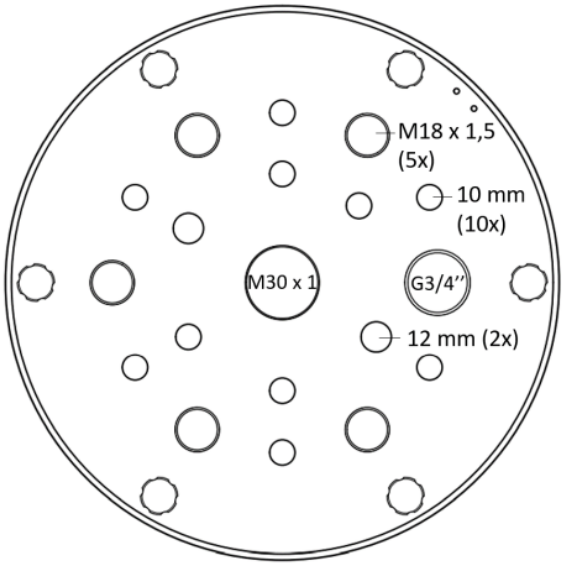
Reactor Type	5 liter, jacketed
Inner Diameter	160 mm
Inner Height (maximum)	250 mm
Liquid Height (working volume)	180 mm
Required Autoclave Space	450 x 260 mm
Req. Autocl. Space with Condenser*	570 x 260 mm*
Overall Height Reactor	330 mm
Total Volume	4.8 liter
Working Volume	3.4 liter
Minimum Working Volume	0.9 liter
H/D Total	1.6
H/D Working Volume	1.1
Jacket Volume	1.8 liter
Volume Indication	Present
Minimum Reactor Pressure	-1 barg
Maximum Reactor Pressure	0.5 barg
Ports in Head Plate	1 * M30 x 1
	1 * G3/4"
	5 * M18 x 1.5
	10 * 10 mm, 2 * 12 mm

*The given dimensions for the required autoclave space are valid for the condenser for microbial applications. The condenser that can be used for cell cultures is 60 mm smaller (less tall).

The head plate also contains two 2 mm holes for level sensor connection.

Z611000520 5-liter Jacketed Reactor

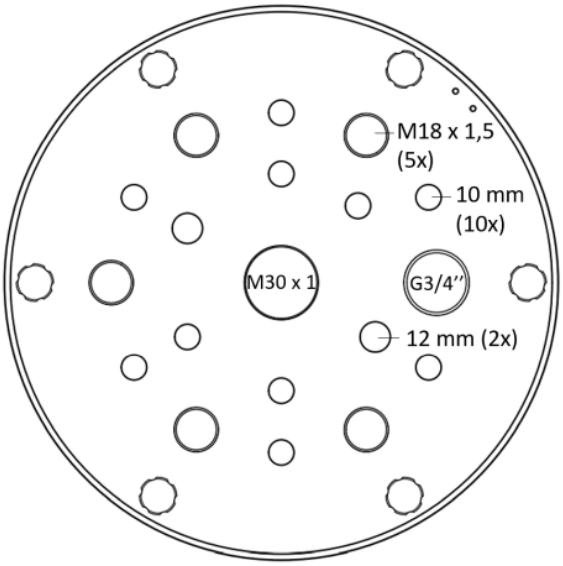
2.3.4 7 LITER REACTOR



Reactor Type	7 liter, dished bottom
Inner Diameter	160 mm
Inner Height (maximum)	350 mm
Liquid Height (working volume)	270 mm
Required Autoclave Space	500 x 260 mm
Req. Autocl. Space with Condenser	610 x 260 mm
Overall Height Reactor	390 mm
Total Volume	6.8 liter
Working Volume	5.4 liter
Minimum Working Volume	1.5 liter
H/D Total	2.2
H/D Working Volume	1.8
Volume Indication	Present
Minimum Reactor Pressure	-1 barg
Maximum Reactor Pressure	0.5 barg
Ports in Head Plate	1 * M30 x 1
	1 * G3/4"
	5 * M18 x 1.5
	10 * 10 mm, 2 * 12 mm

The head plate also contains two 2 mm holes for level sensor connection.

Z611000710 7-liter Dished-bottom Reactor



Reactor Type	7 liter, jacketed
Inner Diameter	160 mm
Inner Height (maximum)	350 mm
Liquid Height (working volume)	270 mm
Required Autoclave Space	540 x 360 mm
Req. Autocl. Space with Condenser	650 x 360 mm
Overall Height Reactor	425 mm
Total Volume	6.8 liter
Working Volume	5.4 liter
Minimum Working Volume	1.5 liter
H/D Total	2.2
H/D Working Volume	1.8
Jacket Volume	2.4 liter
Volume Indication	Present
Minimum Reactor Pressure	-1 barg
Maximum Reactor Pressure	0.5 barg
Ports in Head Plate	1 * M30 x 1
	1 * G3/4"
	5 * M18 x 1.5
	10 * 10 mm, 2 * 12 mm

The head plate also contains two 2 mm holes for level sensor connection.

Z611000720 7-liter Jacketed Reactor

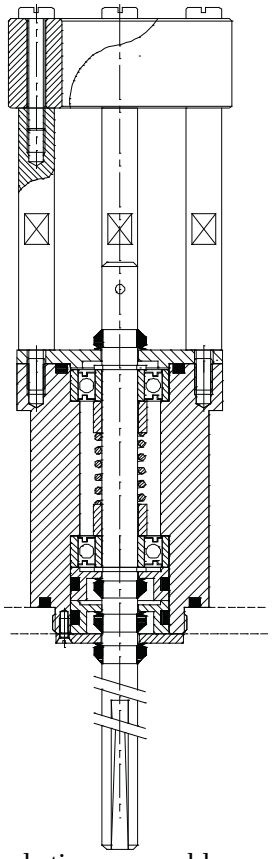
2.4 THE STIRRER ASSEMBLY

Two different stirrer assembly types are available: a lipseal stirrer assembly and a magnetically coupled stirrer assembly .

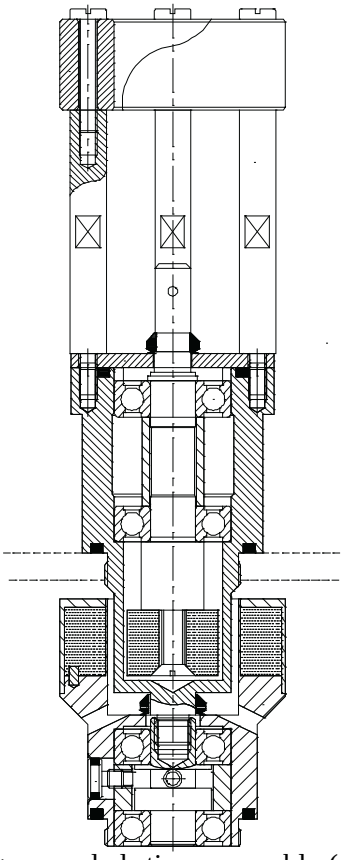
Z81315R003:	Lipseal stirrer assembly 2, 3, 5 liter
Z81315R007:	Lipseal stirrer assembly 7 liter

Z81315MG03:	Magnetically coupled stirrer assembly 2, 3, 5 liter
Z81315MG07:	Magnetically coupled stirrer assembly 7 liter

Material: stainless steel 316.
The assemblies are autoclavable and are designed for long periods of operation with minimum maintenance.



Lipseal stirrer assembly



Magn. coupled stirrer assembly (ex. shaft)

The stirrer shaft of the lipseal stirrer assembly is coupled directly to the stirrer motor. This is the most common way of coupling, whereby contamination-free operation is ensured by the use of viton lipseals.

The magnetically coupled stirrer assembly is especially developed for applications concerning genetic engineering organisms and cell culture. This ensures absolute contamination-free operation since there is no moving seal between the reactor content and the environment.

3 MIXING AND AERATION

For an optimum performance of any biological system, it is necessary to keep the environment of the micro-organisms at optimal conditions. Apart from temperature and medium composition, the two most important factors that effect this environment are the degree of mixing and aeration.

3.1 MIXING

The aim of mixing is to obtain uniform conditions in the working volume of the bioreactor, in order to obtain an optimal mass transfer, to avoid gradients of any of the medium components, and to keep the microcarriers or cells in suspension. In a normally used stirred tank reactor, mixing is accomplished by the impeller. The resulting flow pattern is a function of the impeller configuration, agitation speed, the geometry of the system and gas inflow rate employed.

One aspect of mixing is preventing the cells or microcarriers to settle. In order to achieve this, the fluid velocity must at least be equal to the settling velocity of the particles (v_{sett}), which can be calculated according to Stokes' law (Cherry and Papoutsakis, 1986):

$$v_{sett} = d_p^2 * (\rho_s - \rho_f) * g/(18 * \eta),$$

where: d_p = particle diameter (m),
ρ_s = particle density (kg m^{-3}),
ρ_f = medium density (kg m^{-3}),
g = gravitational constant (m s^{-2})
η = medium viscosity (N m s^{-1}).

Stokes' law only holds for particles whose Reynolds numbers are smaller than 1 (which holds for all normally used biological systems). As the biomass should be homogeneously distributed throughout the reactor, not only must the particles be lifted from the bottom of the vessel but they must also be transported through the whole volume of the reactor.

Generally, the minimum required stirring speed to achieve homogeneous suspension, N_{hs}, is much greater than that to lift the particles from the bottom of the reactor. Buurman et al. (1986) also derived a simple Froude relationship to define homogeneity in a stirred tank reactor (STR), based on the assumption of fluctuating velocity being proportional to the circulation velocity:

$$\rho_f * N_{hs}^2 * D_i^2/(g * (\rho_s - \rho_f) * d_p) = \text{constant}$$

where: D_i = diameter of the impeller (m)

which implies that:

$$N_{hs} \sim 1/D_i$$

The parameter used to describe the medium homogeneity aspect of mixing is the mixing time, which is in fact also the characteristic time for mixing. The mixing time, t_m, is often expressed in the liquid circulation time, t_c. In the case of a stirred tank t_m equals 4 times t_c (Voncken, 1966).
The liquid circulation time in STRs with a Rushton impeller can be calculated according to (Oosterhuis, 1984):

$t_c = V/(0.75 * N * D_i^3)$ where: V = working volume of the reactor (m^3),
N = rotation speed of the impeller (s^{-1}),

If the geometry of the system has to be taken into account in ungassed STRs the following correlation for the mixing time can be used:

$t_m = 1.2/N * (D/D_i)^3 * (H/D) * N_p^{-1/3} * (D_i/H_I)^{2/3}$
where: D = diameter of the reactor (m),
H = liquid height in the reactor (m),
N_p = impeller power number (-),
H_I = height of impeller blade (m).

The impeller power number is a constant for a given system and related to the power input by the stirrer (P_s) in the following way:

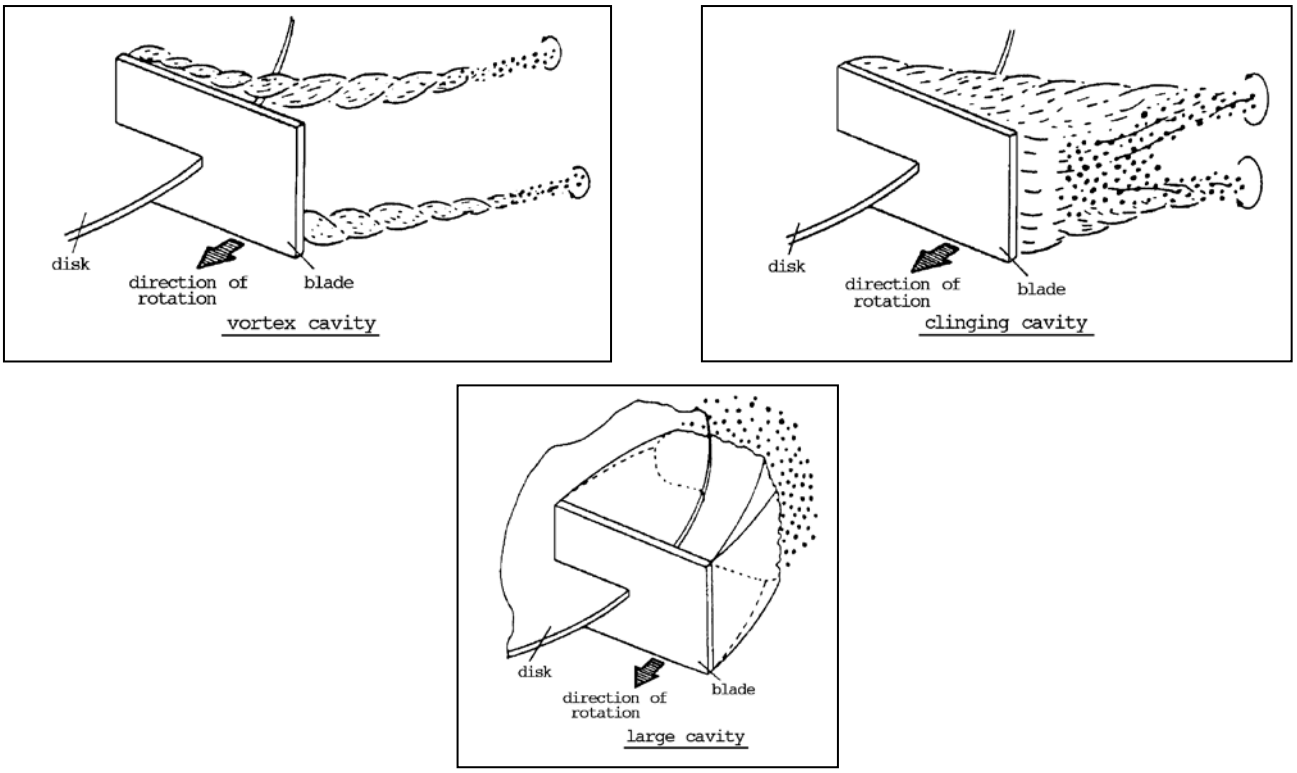
$P_s = N_p * \rho_f * N^3 * D_i^5$

The mixing time in a gassed STR is, as a rule of thumb, twice as high as in an ungassed STR. The resulting mixing time in Applikon's autoclavable bioreactors for mammalian cell and bacterial culture under average operating conditions (ungassed) is given in the following table.

Mixing Times for Applikon Autoclavable Bioreactors									
Reactor Configuration				Mammalian Cell Culture N = 100 rpm, Marine Impellers			Bacterial Culture N = 1000 rpm, Rushton Imp.		
Reactor Volume V (l)	Impeller Diameter Di (mm)	Reactor Diameter Dr (mm)	Liquid Height H (mm)	Impeller Power No Np	Impeller Height Hi (mm)	Mixing Time Tm (sec)	Impeller Power No Np	Impeller Height Hi (mm)	Mixing Time Tm (sec)
2	45	105	175	1.5	28	1.6	2 x 6	11	0.2
3	45	130	200	1.5	28	3.2	2 x 6	11	0.4
5	60	170	200	1.5	37	2.8	2 x 6	15	0.3
7	60	170	300	2 x 1.5	37	3.4	3 x 6	15	0.4
15	74	222	365	2 x 1.5	45	4.3	3 x 6	15	0.5
20	74	222	550	2 x 1.5	45	5.7	3 x 6	19	0.8

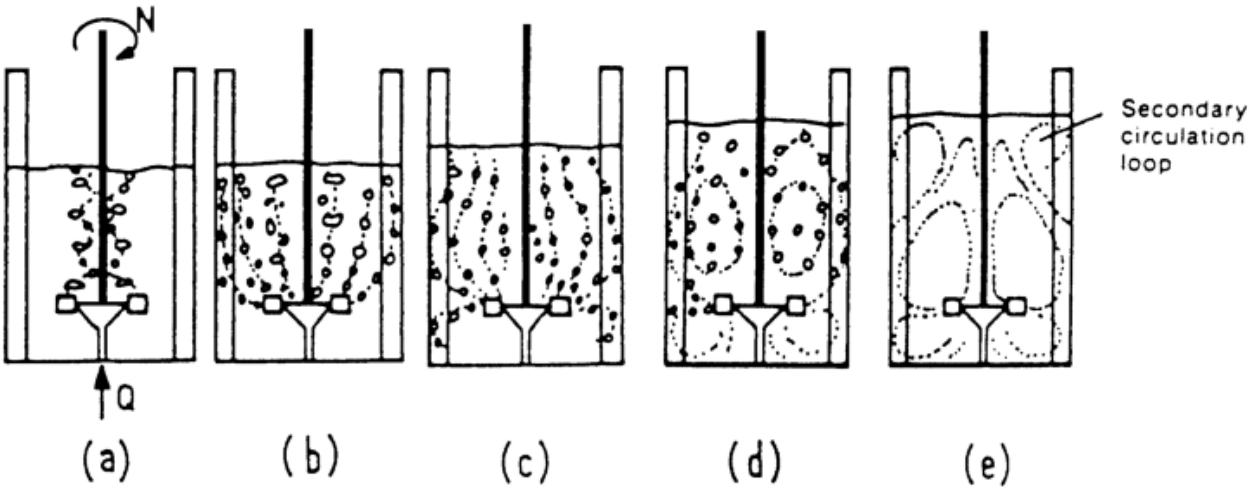
Measurements of the mixing time in a two and three liter reactor have shown to comply well with these correlations (Kakes and Oosterhuis, 1990). It is obvious from this table that the mixing times are very small in relation to the characteristic times that are to be expected for the metabolism (e.g. oxygen uptake rate, substrate consumption rate) of the micro-organisms. Therefore the medium can be looked upon as being ideally mixed at all times during fermentation.

As the bubbles are coalescing behind the impeller and broken up by vortices created by the impeller, the gas will be homogenised in a similar way as the medium and its components. The influence of gas feed on this mechanism is illustrated in the figure below (Warmoeskerken, 1986). Despite this mixing of the gas phase, the cavities behind the impeller blades will decrease the power input to the reactor and thus decrease the oxygen transfer rate to the reactor medium (see chapter 2.2: aeration).



Cavity shape behind Rushton type impellers for small, medium and high gas feed rates.

The operating range of the impeller speed is limited due to the potential damaging effect of the rotating impeller on the micro-organisms. Therefore, it may be impossible to satisfy the criteria for homogeneity. Furthermore, a Rushton type of impeller will only create a homogeneous distribution of bubbles throughout the reactor at relatively high rotation speed (>500 rpm, see figure below, Nienow et al., 1977). Differences in sensitivity to damaging by the impeller and oxygen requirements between bacteria, fungi and mammalian cells have led to the development of various types of impellers. To improve the axially mixing characteristics of the Rushton impeller, impellers with angled blades have been developed. To reduce the damaging effect of the impeller, the (angled) blades are curved in order to create a marine type of impeller for application in mammalian cell or fungi cultures. This impeller also has a good axially pumping capacity which ensures mixing of the complete reactor medium and improves refreshment of the topmost layer of liquid to achieve higher oxygen transfer rates without sparging (see chapter 2.2: aeration).



Gas circulation patterns in aerated stirred vessels at increasing stirring speed (constant gas feed).

3.2 AERATION

Gas-liquid mass transfer in cell culture systems is governed by the solubility of the gas in the liquid medium (Bliem and Katinger, 1988), its molecular diffusivity and the driving force of the gas (which vary with temperature and pressure), and may be described by the expression:

$$OTR = k_l a * (O^* - O_l)$$

where: OTR = oxygen transfer rate,
k_l = liquid phase mass transfer coeff.,
a = gas-liquid interfacial area per unit liquid volume,
O^* = liquid phase oxygen concentration in equilibrium with the bulk gas phase (at 1 atm and 37°C: 0.18 mmol O_2/l).
O_l = actual oxygen concentration of the liquid phase.

The term $k_l a$ represents the volumetric overall mass transfer coefficient and is one of the most common parameters used to describe the efficiency of an aeration system. Bliem and Katinger (1988) compared the typical efficiency of some forms of aeration which are given in the following table.

Efficiencies of different aeration systems		
Typical Oxygen Transfer Rates under culture conditions, using air (cm s^{-1})	$k_l a$ (s^{-1})	Aeration System
10^{-6}	10^{-6}	Static Liquid Surface Aeration
$10^{-6} – 10^{-3}$	$10^{-6} – 10^{-3}$	Stirred Liquid Surface Aeration
$10^{-5} – 8 * 10^{-5}$	$10^{-4} – 10^{-2}$	Silastic Membrane Aeration
$5 * 10^{-5} – 3 * 10^{-4}$	$10^{-4} – 3 * 10^{-4}$	Dynamic Wire Mesh (with agitation)
	$< 7 * 10^{-3}$	Sparged and Stirred Liquid for Cell Culture
	$10^{-2} – 7 * 10^{-1}$	Sparged and Stirred Liquid for Microbial Culture

The $k_l a$-values are strongly influenced by the medium composition. For example, the addition of silicone antifoam can cause the $k_l a$ to decrease drastically whereas the addition of salts to the medium will increase the $k_l a$ (Lavery and Nienow, 1987; Bliem and Katinger, 1988).

For a mammalian cell culture to a density of $5 * 10^9$ cells/l (a typical batch culture), the oxygen requirement is in the range of 5-50 ml O_2/l * h (at 37°C and atmospheric pressure). Therefore, the required $k_l a$ for a typical batch culture operation is likely to be $0.5\text{-}5 * 10^{-4}$ s^{-1}. Static liquid surface aeration is insufficient even for these low requirements (see table on previous page).
For high density cultivation, as reached in long-term perfusion systems, the required $k_l a$ may increase to as much as $0.1\text{-}1 * 10^{-2}$ s^{-1}; this oxygen requirement can only be supplied by sparging.

Sparging is the most efficient way to obtain high k_la values. Most experimental data for k_la values can be estimated by the correlations of Van 't Riet (1979) and Henzler (1982) (adapted for temperature difference):

for coalescing media:
$k_la = (1.022^{(T-20)}) * 0.026 * (P_g/V)^{0.4} * v_s^{0.5}$

for non-coalescing media:
$k_la = (1.022^{(T-20)}) * 0.016 * (P_g/V)^{0.7} * v_s^{0.2}$

where: T = temperature (^oC),
P_g = the gassed power input by impeller (N m s^{-1}),
V = volume (m^3),
v_s = superficial gas velocity (m s^{-1}).

At very low aeration rates P_g will be equal to the ungassed power input P_s (Lavery and Nienow, 1987) but at higher aeration rates the gassed power input has to be calculated according to:

$$P_g \approx 0.5 * P_s = 0.5 * N_p * \rho_f * N^3 * D_i^5$$

The theoretical k_la values from these correlation for Applikon's autoclavable reactors are given in the table below. Measurements of k_la values on a two and three liter scale have shown that the oxygen transfer capacity that can be reached in practice is upto 10 times higher (Kakes and Oosterhuis, 1990). Sparging, however, is known to cause inadmissible shear stress for mammalian cells, will increase the problem of foam formation in bacterial as well as mammalian cultures and is, therefore, limited in its applications.

Applikon Autoclavable Bioreactors: Theoretical Oxygen Transfer Rates

Reactor Configuration			Mammalian Cell Culture N = 100 rpm, Marine Imp., Aeration = 0.05 vvm			Bacterial Culture N = 1000 rpm, Rushton Imp., Aeration = 1 vvm		
Reactor Volume V (l)	Impeller Diameter Di (mm)	Reactor Diameter Dr (mm)	Impeller Power no. Np	Oxygen Transfer		Impeller Power no. Np	Oxygen Transfer	
				Coalesc. k_la (h^{-1})	Non-Coal. k_la (h^{-1})		Coalesc. k_la (h^{-1})	Non-Coal. k_la (h^{-1})
1	45	96	1.5	1	18	6	7	33
2	45	105	1.5	1	12	12	8	37
3	45	130	1.5	1	9	12	7	27
5	60	170	1.5	2	18	12	10	52
7	60	170	3	2	24	18	12	59
15	74	222	3	3	31	18	16	75
20	74	222	3	4	36	18	16	65

Since the required k_la value and admitted aeration flow vary widely from system to system, various ways are used to introduce a gas flow into the system.
For mammalian cell cultures mostly an (oxygen enriched) air overlay is used in combination with an axially pumping impeller. In order to get a large surface area and thus improve the oxygen transfer, reactors with a low H/D value (usually ≈ 1) are used for this application.
Fermentations that require both a low stirrer speed and gas flow need a sintered steel sparger in order to create small bubbles and thus a large area for oxygen transfer.
High gas flow rates can be reached with open pipes, or pipes with several relatively large holes.

All options are available for every Applikon autoclavable bioreactor.

3.3 LITERATURE

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4 OPERATION

4.1 GENERAL

When unpacking the equipment, verify if there is any transport damage and if the reactor is complete (the way you ordered it). Clean all parts with 70% ethanol to remove dust or dirt from shipping. When assembling the reactor, make sure not to damage the threaded ports; always screw in the auxiliaries straight and by hand. Do not use tools to tighten the auxiliaries in the head plate.
Make sure that an O-ring is present between the auxiliary and the head plate in order to ensure sterility

4.2 PREPARING FOR STERILIZATION

Fill the vessel with culture medium, do not exceed the total volume that is specified in chapter 1 (hardware specification). Be sure to leave enough volume for additions after sterilization (e.g. inoculum, separately sterilized nutrients, etc.). Fasten the six mill nuts crosswise by hand. Verify the functioning of the sensors (calibrate the pH sensor). Insert the Level probe as far as possible into the vessel and fasten it (after sterilization, the position of this probe can be adjusted, upward to the desired height).
Verify the mounting of all nipples and other auxiliaries. Make connections for liquid additions, air in and air out with silicone tubing or other suitable sterilizable material. Use appropriate filters for air in and air out. To avoid wetting of the inlet filter during sterilization, use a clamp to close the tubing between the head plate and the filter. Close all other connections (except the air out) air-tight with a hose and a hose clamp. Close all open tubing ends with cotton and cover the ends with sterilizable foil or paper.
Make sure that the vessel is not completely closed, since pressure differences during sterilization may damage the reactor or the probes. Use the air outlet filter to maintain pressure equilibrium in- and outside the reactor. The heat exchanger should be empty during sterilization.

4.3 STERILIZATION

The bioreactor with all accessories (except the stirrer motor) can be placed in an autoclave.

The autoclave should stay at 121°C for at least 20 minutes in order to kill all organisms and thermo-resistant spores. After sterilization, let the autoclave cool down without opening it, until the temperature is below 90°C. When the temperature in the autoclave has dropped below 90°C, it can be opened to allow it to cool down further. This cooling procedure should be performed to avoid low pressure in any part of the reactor system. Low pressure in tubing might result in contamination when the tube is connected to a peripheral device.

In case of a jacketed reactor, the sterilization interval might need to be increased, since the empty jacket has a poor heat transfer capacity.
If the medium cannot stand a longer interval, you can fill the jacket with water to improve the heat transfer (connect and close the lower tube fitting, do not close the upper one).

4.4 INSTALLATION

Put the reactor as near as possible to the control equipment and connect all sensors to it. Make the following connections:

- the heat exchanger to the thermo circulator
- the air inlet pipe to the flow console
- cooling water to the condenser

Aseptically connect the sterile fluids (acid, base, anti-foam, etc.) that have to be added to the medium, aseptically to the inlet pipes.

To improve heat transfer between the thermometer pocket and the Pt100-sensor, fill the thermometer pocket with water or silicone oil. This will decrease the dead time of the sensor and will make temperature control more accurate.
When operating at higher temperature, silicone oil has the advantage of a lower vapour pressure (less evaporation).

4.5 PREPARING FOR OPERATION

After connecting all cables and tubing, adjust the set points of the controllers to the desired value (temperature, pH, DO_2, etc.).
Switch on the thermo circulator, stirrer motor, acid and/or base pumps and gas flow.
When temperature, pH etc. have reached their set point (and are stabilized), the bioreactor is ready for inoculation.

4.6 INOCULATION

There are several ways to inoculate. Two methods that are commonly used are described here:

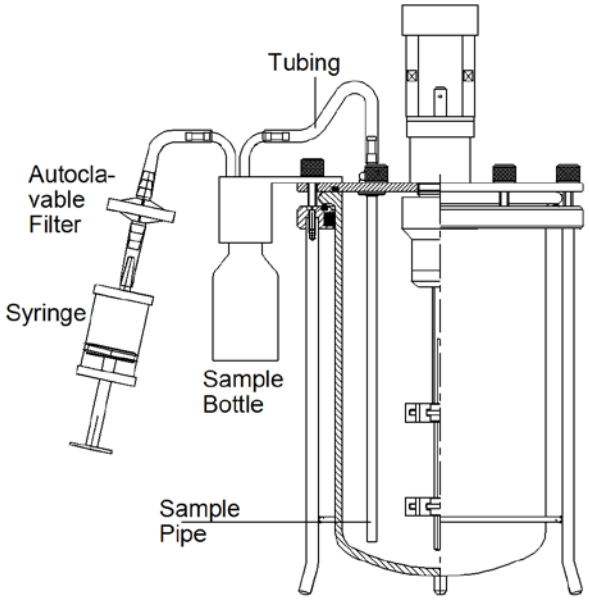
1. Fill a sterile flask, to which a sterile hose is connected, aseptically with inoculum. The other end of the hose should be connected to a sterile needle. Turn off aeration, pierce the needle through the septum and transfer the inoculum to the reactor by gravity feed or by using a pump.
2. In this case a sterile syringe is filled with inoculum. The needle is pierced through the septum and the inoculum is pushed into the reactor (this method is suitable for inoculum volumes smaller than 100 ml).

Other methods of inoculation can be used if they are performed aseptically.

4.7 ACTIONS DURING FERMENTATION / CULTIVATION

4.8 SAMPLING

To sample a fermentation broth, a sample pipe is needed to which a sample system is attached. Connect a syringe to the tubing that is connected to the "out barb" of the sample system. In this tube there should be a filter or cotton.
Pull the piston of the syringe to create a vacuum in the sample bottle. As a result, the fermentation broth is pulled into the sample bottle. Replace the sample bottle aseptically with a sterilized empty one.

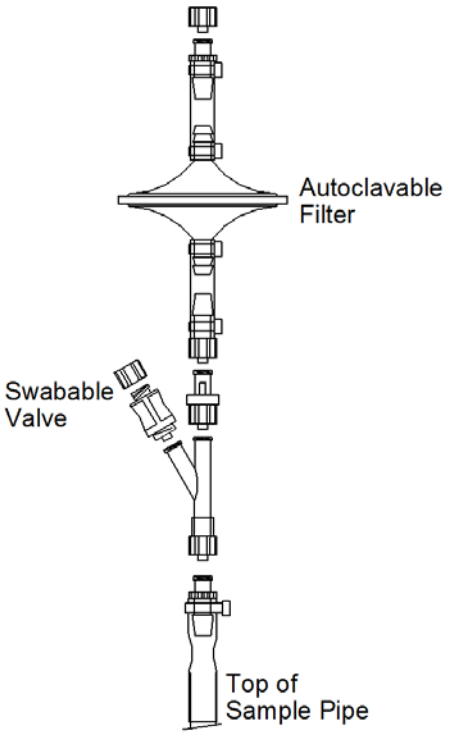


4.8.1 SINGLE USE SAMPLE SYSTEM

This system is mounted on the standard autoclavable sample pipe and is autoclaved with the bioreactor. When a sample needs to be taken from the bioreactor, a syringe is placed on the filter to push air through the sample pipe in order to clear it.
Next, a sterile syringe is placed on the swabable valve and a sample can be drawn into the syringe.

The swabable valve must be cleaned with 70% ethanol before and after sampling.

Using this sample system has the benefit that no sample bottles need to be autoclaved and no flames are required to maintain sterility. Since the swabable valve can only be used for 10 to 15 samples, the sample system should be replaced after each cultivation.



4.8.2 ADDITIONS

If extra substrate is needed in the culture during fermentation, it can be added as described under "inoculation".
Make sure there is enough space left in your vessel for additions.

4.9 DECONTAMINATION AND HARVESTING

Depending on the type of organism in the broth, it may be necessary to decontaminate the bioreactor and its auxiliaries (e.g. by sterilizing it again). Refer to the relevant Standard Operating Procedures.

After decontamination, the reactor can be harvested. Refer to the relevant Standard Operating Procedures.

5 CLEANING

After finishing the fermentation process, the glass and stainless steel parts should be cleaned thoroughly. Use hot water, 70% ethanol or other suitable cleaners to clean all parts. Never use abrasive materials to clean the metal parts.

5.1 CLEANING THE SENSORS

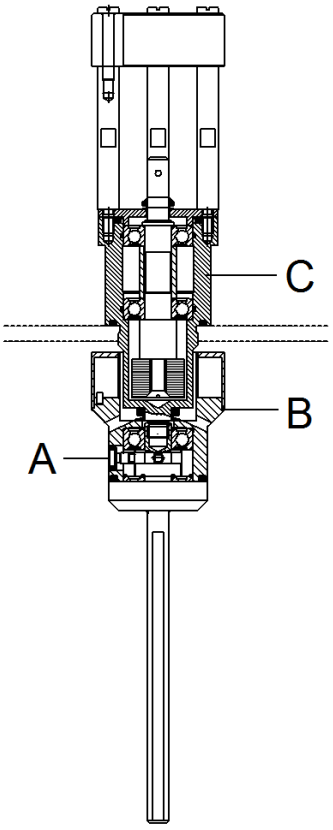
- Remove the pH and DO sensors.
- Clean the pH and DO sensors thoroughly. Make sure that no remains of the broth are left behind on the sensor surface. Refer to the cleaning instructions in the user manual of these sensors.
- Store the pH sensor in a Kcl (c=3M) solution.
- The DO sensor may be stored dry.

5.2 CLEANING THE BIOREACTOR

- Fill the reactor with a NaOH (c=0.1M) solution.
- Activate the stirrer at high speed. Visual check for dissolution of foam, debris and other contamination in the reactor. This takes approx. 30 minutes.
- Drain the reactor.

5.3 DISASSEMBLING THE BIOREACTOR

- Remove the head plate including the stirrer assembly.
- Remove the auxiliaries.
- Remove the baffles (can be omitted in case of the 1L reactor).
- Remove the impellers from the stirrer shaft.
- Disassemble the magnetically coupled stirrer (lipseal stirrers do not need to be disassembled).
  -Head plate including stirrer assembly are removed from the reactor
  -Turn dismantling screw (A) as far as possible into the housing in order to lock the shaft
  -Loosen the rotor part (B) by turning it counter clockwise
  - Pull-off the rotor part. Beware of the magnetic field
  - Remove the motor coupling part (C) from the head plate by turning it counter clockwise



5.4 CLEANING THE PARTS

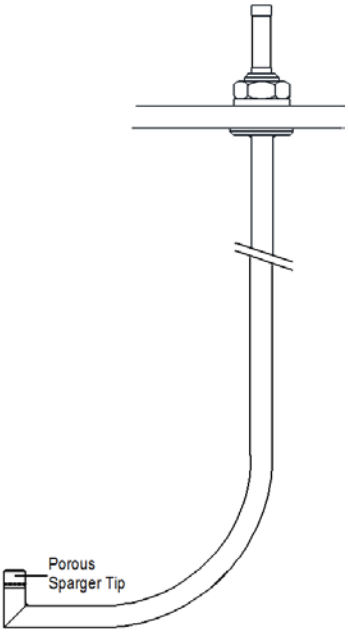
- Clean all parts by using hot water, 70% ethanol or other suitable cleaners.

Caution:
Never immerse the rotor into any (cleaning) liquid when the stirrer shaft is not in place (ball bearings will be damaged).

- Let all parts dry.

5.5 CLEANING THE POROUS SPARGER

- Depending on the type of medium composition (presence of proteins and/or peptides), cleaning the Porous Sparger may require a special procedure:
- Remove the porous sparger tip from the air inlet pipe
- Soak it overnight in a solution of pepsin (c = 10 mg/ml) / HCl (c = 0.01 M)
- Use ultra-sonic cleaning with the following solvents:
  d.i. water
  ethanol (c = 70%)
- Store the Porous Sparger dry until it is required for use in the next run.



5.6 RE-ASSEMBLING THE BIOREACTOR

Re-assemble and mount the head plate (also refer to chapter 3).
Take care not to damage or forget any O-rings, since this can cause contamination during the next run.

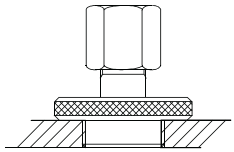
When the reactor is frequently used, it is advised to replace the O-rings of the auxiliaries twice a year.

6 AUXILIARIES

In this chapter, the head-plate-auxiliaries for the 2 - 7 liter bioreactors are listed.

6.1 SAMPLING

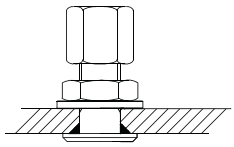
Assembly holder 6 mm tube for M18 x 1.5 port:



This device fits into a M18 x 1.5 port and can be used to hold any 6 mm (O.D.) tube. The insertion length of this tube can be varied; additions to or sampling from the culture fluid can take place at any level inside the reactor.

Z811302015 2 - 7 Liter reactor, M18 x 1.5 port

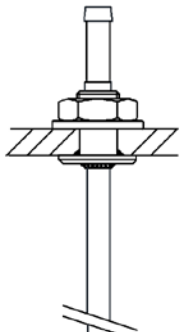
Assembly holder 6 mm tube for 10 mm port:



This device can be inserted into a 10 mm port and can be used to hold any 6 mm (O.D.) tube. The insertion length of this tube can be varied; additions to or sampling from the culture fluid can take place at any level inside the reactor.

Z81320AH00 2 - 7 liter reactor, 10 mm port

Sample pipe (fixed length):

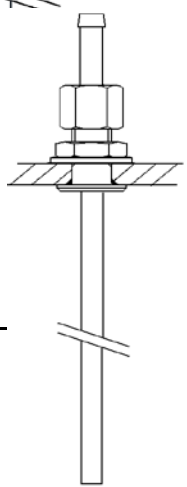


This assembly is used to sample the culture fluid.

Tube diameter (O.D.): 6 mm (10 mm port)
9.5 mm (12 mm port)

Insertion length: 213 mm (for 2, 3 and 5 l reactors)
325 mm (for 7 l reactor)

Z81319MB03 2 - 5 liter reactor, 10 mm port
Z81319MB05 2 - 5 liter reactor, 12 mm port
Z81319MB07 7 liter reactor, 10 mm port
Z81319MB08 7 liter reactor, 12 mm port



Sample pipe (height adjustable):

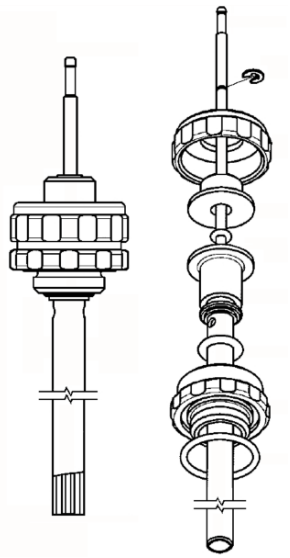
The height adjustable sample pipe assembly consists of:
- an assembly holder for 6 mm tubes and
- a O.D. 6 mm sample tube.

Tube diameter (I.D.): 4 mm.
Maximum insertion length: 232 mm (2 - 5 liter reactor)
320 mm (7 liter reactor)

With this device, the culture fluid can be sampled at any desired level.

Z81319MB04	2 - 5 liter reactor, 10 mm port
Z81319MB06	7 liter reactor, 10 mm port

Chemostat tube:



The chemostat tube is used in continuous fermentation.
This device is designed to achieve a constant level in the reactor.
Liquid is taken out of the reactor through the height adjustable inner tube.
This inner tube is shielded from the reactor by an outer tube to avoid the influence of foam and surface irregularities on the liquid level.

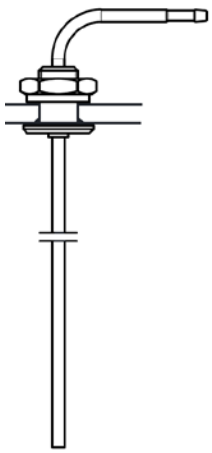
Diameter outer tube: O.D. = 8 mm
I.D. = 6 mm

Diameter inner tube: O.D. = 3.18 mm
I.D. = 1.4 mm

Insertion length: 156 mm (2 - 5 liter reactor)
(outer tube) 306 mm (7 liter reactor)

Z813060002	L = 157 mm, M18 x 1.5 port
Z813060003	L = 306 mm, M18 x 1.5 port

Sample pipe (I.D. = 1.5 mm):



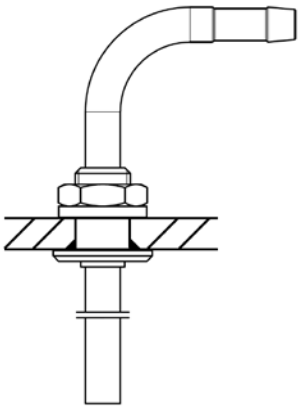
This sample pipe is designed for the sampling of small volumes.
The internal diameter of the pipe guaranties a minimum dead volume. The sample pipe fits into a 6 mm (baffle) port or into a 10 mm port, depending on type number.

Diameter: 1.5 mm

Z81319MB13	2 and 3 liter reactor, 6 mm port
Z81319MB15	2 - 7 liter reactor, 10 mm port

Drain tube:

The drain tube is used to take relatively large samples from the culture and to drain bio-reactor after finishing the process.

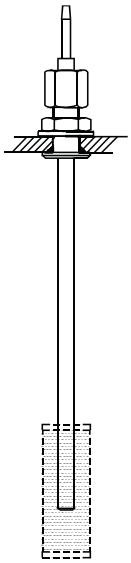


Diameter:	O.D. = 6.35 mm I.D. = 4.53 mm
Z81319MB14	2 - 5 liter reactor, 10 mm port

Sample pipe for screens:

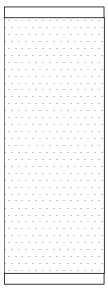
The sample pipe for screens has a very small dead volume. The height adjustable sample pipe can be used with or without a sample screen.
If it is used without sample screen, the small dead volume of the pipe guarantees samples from the culture that are representative for the reactor contents.

If a sample screen is used at the end of this pipe, cell free samples can be drawn from the culture. Sample screens are available in several pore sizes (see below).



Z81319MB09	2 - 5 liter reactor, 10 mm port
Z81319MB11	7 liter reactor, 10 mm port

Sample screen:

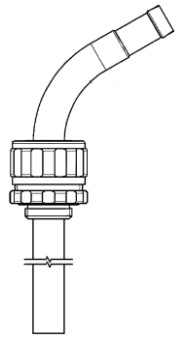


Available pore sizes:

Z811303010	13 µ sample screen
Z811303011	25 µ sample screen
Z811303012	76 µ sample screen
Z811303013	105 µ sample screen

Sample pipe I.D. 10 mm:

This sample pipe is designed for sampling cultures with flocculating organisms (in this case a sample pipe with a small diameter will ruin the flocks and the pipe will be clogged).
The shear forces inside this sample pipe are nearly negligible.

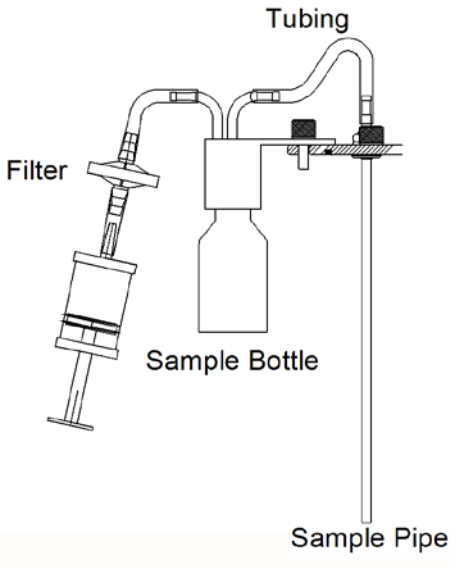


The sample pipe can be fitted in a 12 mm I.D. pH/mV nipple.

Z813190022	2 - 7 liter reactor, pH/mV nipple

Sample system:

The sample system with a 60 (or 30) ml glass bottle can be mounted onto the head plate of the bio-reactor.
This system, completed with a syringe and connected to the sample pipe (tubing), provides your bio-reactor with an easily operated sampling device.
This system, completed with a syringe and connected to a sample pipe (tubing), provides your reactor with an easily operated sampling device.

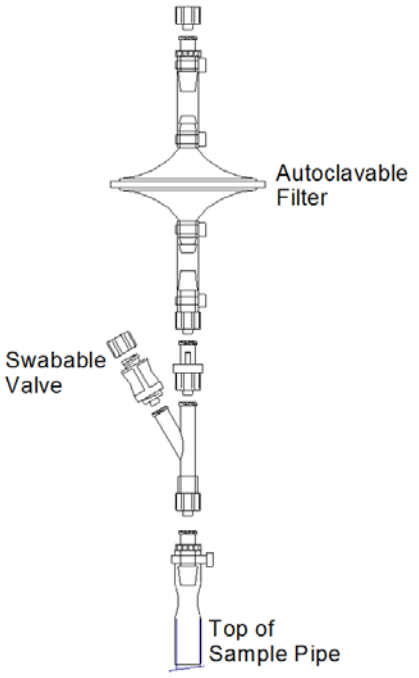


Z81207SS02	Sample System (all reactors)
Z81207BT30	Sample bottle 30 ml
Z81207BT60	Sample bottle 60 ml

Single Use Sample System:

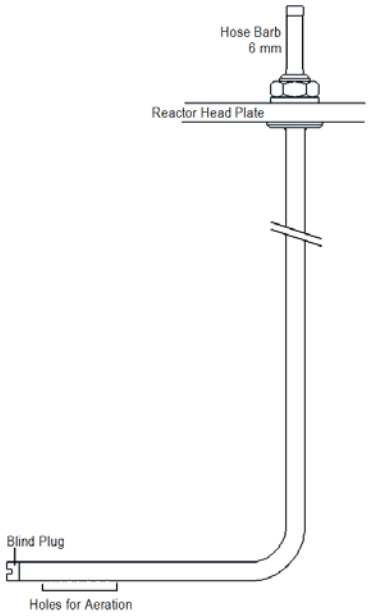
The single use sample system includes 5 sample systems and 25 syringes of 10 ml each.
This system is mounted on the sample pipe in the reactor.

Z850000010	Single Use Sample System (5 pieces)



6.2 AERATION

Air-inlet (sparger) pipe:



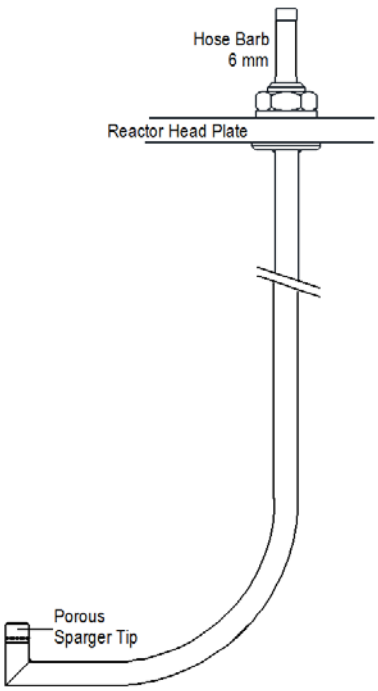
To meet the oxygen demand of a culture, a sterile gas stream can be sparged through the culture, using an
air-inlet pipe.
This pipe can be applied when high gas flow rates are
required, since this pipe causes hardly any pressure drop.

The holes in this pipe are located at the bottom to make sure that medium will be driven out by the gas stream.

Insertion length: 236 mm (2 - 5 liter reactor)
330 mm (7 liter reactor)

Z81318L002	2 liter reactor, 10 mm port
Z81318L003	3 liter reactor, 10 mm port
Z81318L005	5 liter reactor, 10 mm port
Z81318L007	7 liter reactor, 10 mm port

Air-inlet pipe with porous sparger:

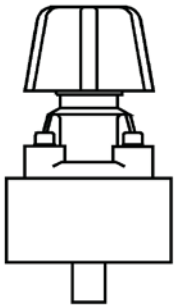


In cell culture fermentations, high gas flow rates (causing shear forces) might damage the cells.
To be able to meet the oxygen demand of the cells at lower gas flow, the exchange-surface must be increased.
This can be achieved by using this sparge pipe.
The sintered metal tip produces tiny air bubbles for optimum gas distribution.

Insertion length: 238 mm (2 - 5 liter reactor)
330 mm (7 liter reactor)

Z81318L004	2 liter reactor, 10 mm port
Z811303005	3 liter reactor, 10 mm port
Z81318L006	5 liter reactor, 10 mm port
Z81318L008	7 liter reactor, 10 mm port
Z811303008	Porous sparger for air-inlet pipe

Tuning valve:



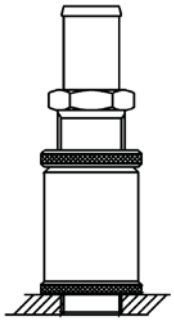
The tuning valve can be installed on top of the stainless steel air-outlet condenser in order to create a small over-pressure in the reactor.
This has the following advantages:

- risk of contamination is reduced,
- oxygen transfer to the medium is increased,
- sampling the culture is eased.

For safety reasons it is strongly advised to use this tuning valve in combination with the pressure relief valve (listed below).

Z811302020 2 - 7 Liter reactor

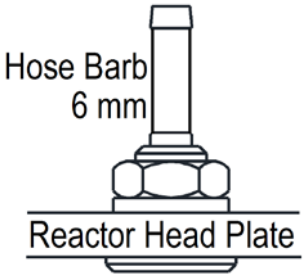
Pressure relief valve:



When over-pressure is applied in the (glass) Applikon 2 - 7 Liter bio-reactors, it is advised to install this relief valve.
The pressure at which the relief valve will open can be adjusted manually.

Z811302050 2 - 7 Liter reactor, M18 x 1.5 port

Air-outlet pipe:

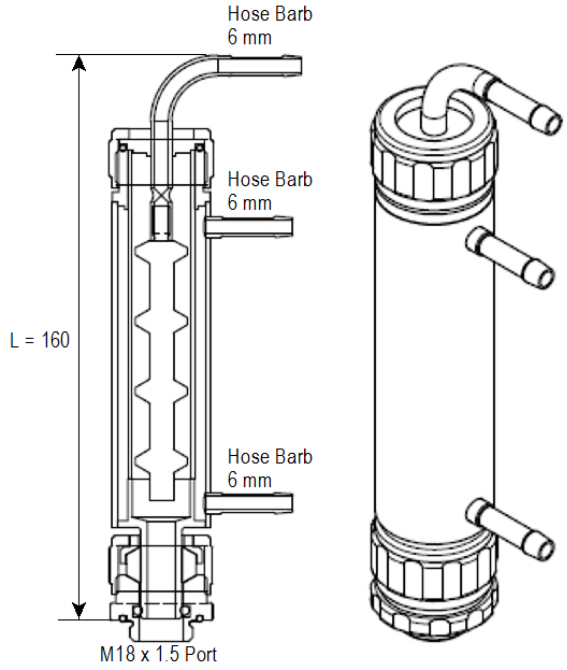


The air-outlet pipe can be used for either gas outlet or gas overlay.
The latter use is for head space aeration (separate from or in combination with sparging gas through the culture).

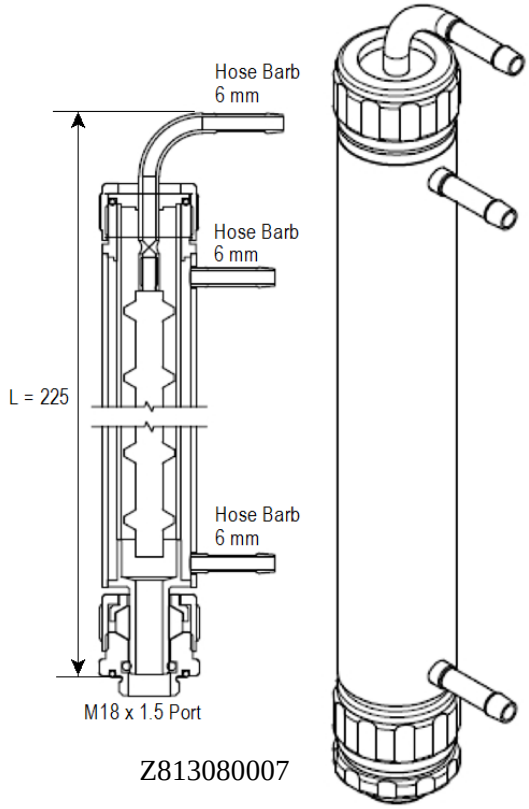
Z81308LU02 2 - 7 liter reactor, 10 mm port

Gas-outlet condenser:

Working at elevated temperatures and using aeration of the culture might cause too much evaporation during fermentation, causing an increase of nutrient concentration and a decrease in volume. An gas-outlet condenser can prevent this.
The stainless steel gas outlet condenser is available in three different versions, both fitting in the M18 x 1.5 port of the reactor head plate:



Z813080002



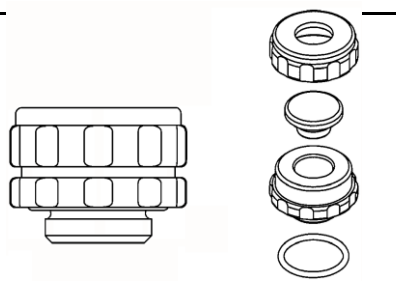
Z813080007

Z813080002	Baffled Condenser for M18 x 1.5 port, L = 160 To be used in the 2 and 3L reactors and the 5L for Cell Culture
Z813080007	Baffled Condenser for M18 x 1.5 port, L = 225 To be used in the 7L reactors (and the 5L for Microbial applications)

If the reactor plus affixed condenser is too tall for the autoclave, a flexible coupling can be inserted between reactor and condenser. During the cultivation, the condenser is held upright by the condenser support. During autoclaving, the flexible coupling between reactor and condenser allows the condenser to be bent to a horizontal position.

Z81308C005	Flexible Coupling Condenser for 2 – 7L Reactors

6.3 ADDITION



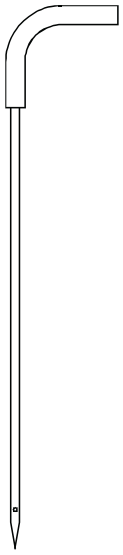
Septum holder:

The septum holder is equipped with a silicone rubber septum and can be used as a universal addition port by piercing it with one or more needles.

Z813020002 2 - 7 Liter reactor, M18 x 1.5 port

Needle for septum:

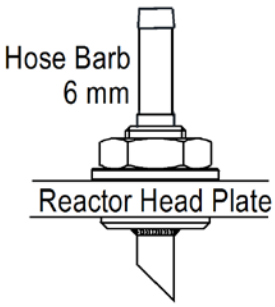
The needle is used to pierce the septum and to add a fluid or gas to the culture.



Z81309IN02 Needle for septum

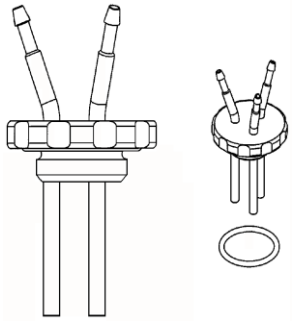
Addition pipe 10 mm port:

This addition pipe can be used to add fluids or gasses to the reactor.



Z81324MT02 2 - 7 liter reactor, 10 mm port
Z81324MT05 2 - 7 liter reactor, 12 mm port

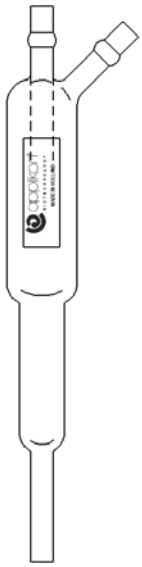
Medium inlet triple:



The medium inlet triple allows you to equip one M18 x 1.5 port with three addition ports (e.g. for acid, alkali and anti-foam addition). This device can be used to expand the number of entries beyond the number of ports in the head plate.

Z813240003 2 - 7 Liter reactor, M18 x 1.5 port

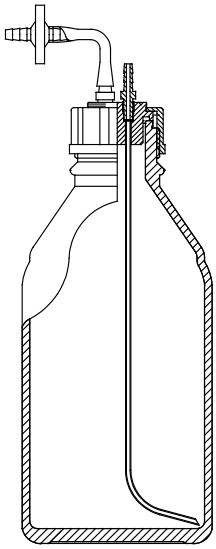
Liquid entry system:



When running a continuous culture, backgrowth of organisms into the medium container must be prevented.
The liquid entry system uses a sterile gas flow to transfer the fresh medium to the reactor; in this way, direct contact between the culture and the medium storage container does not exist.
The liquid entry system fits into the pH/mV nipple (Z81300N002).

Z81309IN03 2 - 7 Liter reactor, pH/mV nipple

Liquid addition bottle:

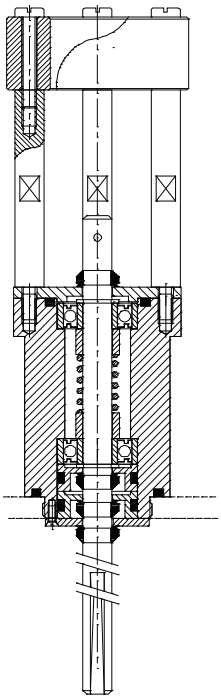


The liquid addition bottles are available in the following sizes:

Z811301918 Liquid Addition Bottle, 0.25 L, 1.5 barg
Z811301919 Liquid Addition Bottle, 0.5 L, 1.5 barg
Z811301920 Liquid Addition Bottle, 1 L, 1.5 barg
Z811301911 Liquid Addition Bottle, 2 L, 1,5 barg
Z811301912 Liquid Addition Bottle, 5 L, 1.5 barg

The liquid addition bottle comes with an air-inlet filter.

6.4 MIXING

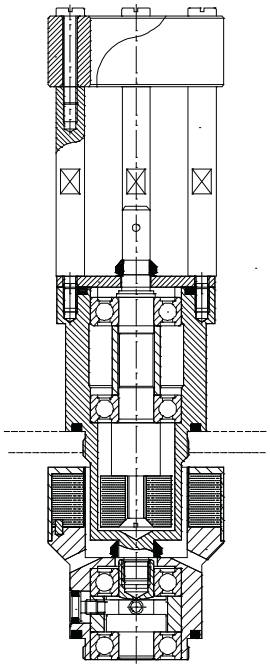


Two types of stirrer assemblies are available for insertion in the central M30 x 1 port of the head plate:

1. The lipseal stirrer assembly:

Material:	Stainless Steel
Diameter shaft:	8 mm

Z81315R002	1 liter reactor, M30 x 1 port
Z81315R003	2 - 5 liter reactor, M30 x 1 port
Z81315R007	7 liter reactor, M30 x 1 port

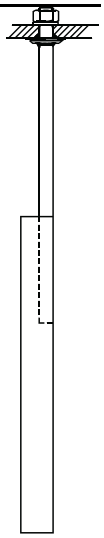


2. The magnetically coupled stirrer assembly:

Material:	Stainless Steel
Diameter shaft:	8 mm

Z81315MG02	1 liter reactor, M30 x 1 port
Z81315MG03	2 - 5 liter reactor, M30 x 1 port
Z81315MG07	7 liter reactor, M30 x 1 port

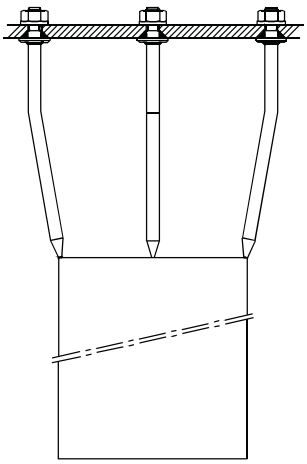
Baffle assembly:



Baffles are used to increase the mixing efficiency (without baffles, the medium flow can become laminar, causing poor mixing efficiency and mass transfer). The baffles are mounted near the reactor wall for optimum mixing performance.
The baffle assembly consists of one baffle and mounting material.
Normally three baffles are used to create maximum mixing efficiency.

Insertion length:	220 mm (for 2 and 3 liter reactor)
	223 mm (for 5 liter reactor)
	317 mm (for 7 liter reactor)
Z81326KS03	2 and 3 liter reactor, 6 mm port
Z81326KS05	5 liter reactor, 10 mm port
Z81326KS07	7 liter reactor, 10 mm port

Draught tube:



The draught tube is used to obtain maximum mixing efficiency and optimum oxygen mass transfer by enforcing an axial flow pattern in the reactor.
The draught tube must be used in combination with a marine impeller (the vortex type for foam killing, the scoping type in case a vortex or gas bubbles in the culture liquid are not allowed).

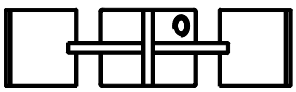
Insertion length:	220 mm (3 liter reactor)
Tube length:	120 mm

Draught tubes for other reactors: on request.

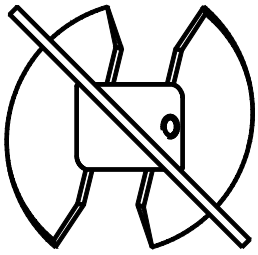
Z81334DT03	3 liter reactor, 3 x 6 mm port

Impellers:

The following impellers are available for the Applikon stirrer assemblies (lipseal and magnetically coupled):

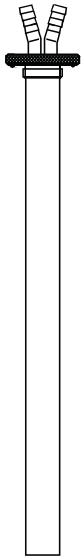


Z81313R602 Rushton impeller, 6 bladed, D = 45 mm
Z81313R645 Rushton impeller, 6 bladed (vortex), D = 45 mm
Z81313R607 Rushton impeller, 6 bladed, D = 60 mm



Z81314RC02 Marine impeller (vortex), D = 45 mm
Z81314RC03 Marine impeller (scoping), D = 45 mm
Z81314RC07 Marine impeller (vortex), D = 60 mm
Z81314RC08 Marine impeller (scoping), D = 60 mm

6.5 HEAT EXCHANGERS

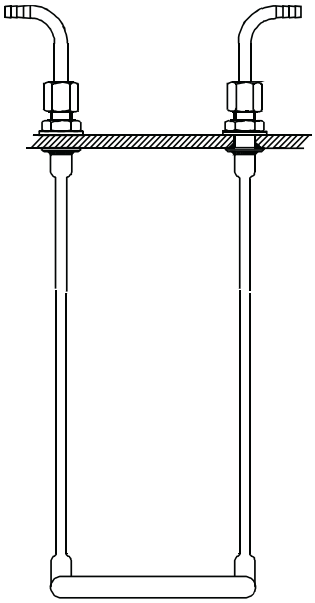


Two different types of heat exchangers are available.

1: fitting into a M18 x 1.5 port. This heat exchanger can be used in the 2 - 5 liter reactors.

Insertion length:	226 mm
Z81317KV03	2 - 5 liter reactor, M18 x 1.5 port

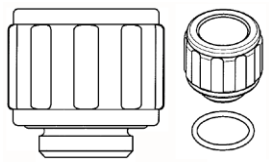
2: fitting two 10 mm ports. This type of heat exchanger is available for the 5 and 7 liter reactor.



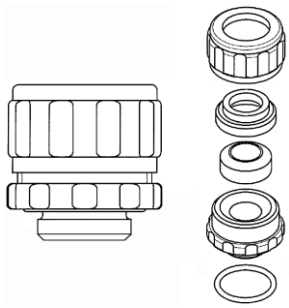
Insertion length:	210 mm (5 liter reactor) 315 mm (7 liter reactor)
Z81317HE05	5 liter reactor, two 10 mm ports
Z81317HE07	7 liter reactor, two 10 mm ports

6.6 SENSOR HOLDERS

Sensor Holder ID = 12 mm for M18 x 1.5 port:
This nipple fits into a M18 x 1.5 port and can accommodate:
- sensors (pH, dO_2, etc.)
- liquid entry system
- glass condenser
- or other 12 mm (O.D.) devices.



Z813000011



Z813000002

Z813000011 Sensor Holder ID = 12 mm, M18 x 1.5 port, PG 13.5
Z813000002 Sensor Holder Height Adjustable ID = 12 mm, M18 x 1.5 port

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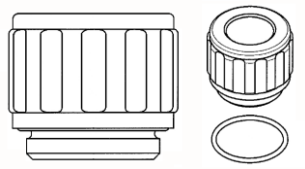
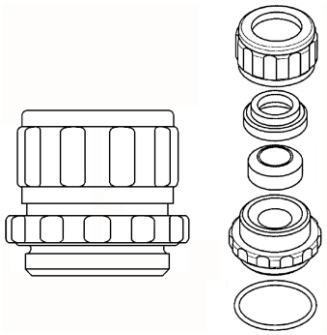
Sensor Holder ID = 12 mm for G3/4" port:
This nipple can be used to fit a pH or mV electrode or
any other device with an OD of 12 mm (fixed height) to the G3/4" port.



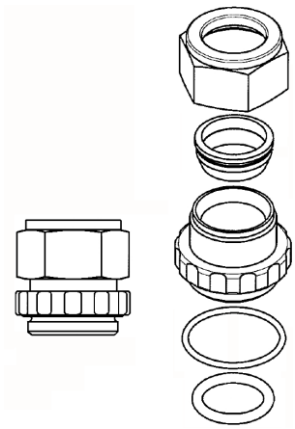
Z813000021 Sensor Holder, G3/4" port, PG 13.5

Sensor Holder height adjustable ID = 12 mm for g3/4"port:



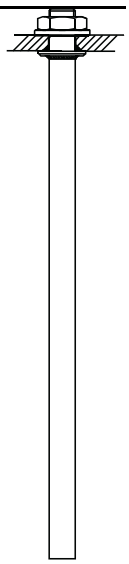
Z813000005 Sensor Holder height adjustable ID = 12 mm, G3/4"port

Nipple ID = 19 mm for G3/4" port:
Every 2 - 7 liter head plate is equipped with a G3/4" port for the electrode holder for DO sensors (OD = 19 mm) and Bio Mass sensors. The sensor is height-adjustable.



Z811303002 Sensor Holder height adjustable ID = 19 mm, G3/4"port

Thermometer pocket:



Temperature measurement (and control) is very important in bio-technology. The thermometer pocket allows you to insert a temperature probe (Pt-100 or mercury thermometer) in the reactor. Fill the thermometer pocket with water or silicone oil in order to improve thermal contact between the culture and the probe.

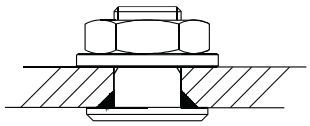
Insertion length:	200 mm (2 - 5 liter reactor) 300 mm (7 liter reactor)
Z81323TP03	2 - 5 liter reactor, 10 mm port
Z81323TP07	7 liter reactor, 10 mm port

Available sensors (diameter of the pH and dO_2 electrodes = 12 mm):

Z001023551	Sensor pH+ (annular junction), L = 235 mm for 1 - 5 liter reactor, PG 13.5
Z001032551	Sensor pH+ (annular junction), L = 325 mm for 7 liter reactor, PG 13.5
Z100200010	Cable pH sensor L = 2 m
Z010023525	Sensor DO (low drift), L = 235 mm for 1 - 5 liter reactor
Z010032525	Sensor DO (low drift), L = 325 mm for 7 liter reactor
Z100200012	Cable DO sensor L = 2 m
Z034150010	Sensor temperature (Pt-100), L = 200 mm
Z71205AF02	Sensor foam for 1 liter reactor
Z71205AF03	Sensor foam for 2 - 7 liter reactor
Z103150010	Cable foam sensor autoclavable reactors

6.7 BLIND STOPPERS

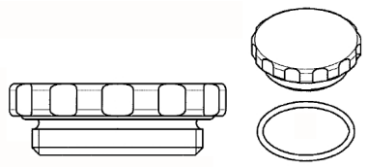
Blind stopper 6, 10 and 12 mm ports:



These blind stoppers can be used to blind unused ports in the head plate; the assemblies are fitted into the head plate from the inside of the reactor, leaving only a flat surface in the reactor.

Z81321BP03	2 and 3 liter reactor, 6 mm port
Z81322BP03	2 - 7 liter reactor, 10 mm port
Z81322BP08	2 - 7 liter reactor, 12 mm port

Blind stopper threaded ports:



The following blind stoppers are available for the threaded ports:

Z813010004	2 - 7 Liter reactor, G3/4" port
Z813010003	2 - 7 Liter reactor, M30 x 1 port
Z813010002	2 - 7 Liter reactor, M18 x 1.5 port

6.8 START-UP KIT

Two different Start-up Kits with parts for the autoclavable 2 - 7L reactors are available:

- For the 2 and 3L reactors,
- For the 5 and 7L reactors.

The table below lists the content of the Start-Up Kit for the 2 and 3 liter reactors:

Part Number	Part Description	Quantity Per Assembly
V3MA000031	CLAMPING RING FOR PH-NIPPLE VITON	2
V1S4ARP124	O-RING ID 31.42X2.62 SILICONE	2
V1S4ARP111	O-RING ID 10.77X2.62 SILICONE	4
V1S4ARP023	O-RING ID 26.70X1.78 SILICONE	2
V1S4135X40	O-RING ID135.00X4.00 SILICONE	2
V1S4085X25	O-RING ID 8.50X2.50 SILICONE	12
V1S4057X19	O-RING ID 5.70X1.90 SILICONE	6
V1S4026X19	O-RING ID 2.60X1.90 SILICONE	5
V1S4022X16	O-RING ID 2.20X1.60 SILICONE	2
V1S4020X20	O-RING ID 20.00X2.00 SILICONE	10
V1S400PDR3	RUBBER SEPTUM-SMALL SILICONE GREY	5
V1S4004X10	O-RING ID 4.00X1.00 SILICONE	5
V1S1302007	LIPSEAL FOR ST.ASS.V- 7S FPM BLACK - NON FDA	4
V1S1302001	LIPSEAL FOR ST.ASS.V-10A FPM - FDA COMPLIANT	2
V0W3300006	TWIST LOCK 22.9 - 25.4MM	10
V0W3300005	TWIST LOCK 14.0 - 16.5MM	10
V0W0700001	TUBING CLAMP	6
V0W0530005	REDUCER MALE-MALE 1/16"-3/16"	3
V0W0530004	REDUCER MALE-MALE 1/8"-3/16"	1
V0W0430002	T-CONNECTOR 1/8"-1/8"-1/8"	2
V0W0430001	T-CONNECTOR 3/16"-3/16"-3/16"	1
V0R9900101	ALLEN KEY 1.5 MM	1
V0R9900007	COMP. STORAGE BOX 312x238x51MM, 21 DIVIDERS	1
V0R1451010	CABLE TIE BLACK 92X2.3 MM	25
V0N0000093	KWIK CLAMP PA HOSE OD 9.1-11.0MM	12
V0N0000091	KWIK CLAMP PA HOSE OD 6.5-8.2MM	4
V0N0000090	KWIK CLAMP PA HOSE OD 5.5-6.8MM	6

Z81100AK12 Start-Up Kit II for autoclavable reactors 2 and 3L

The table below lists the content of the Start-Up Kit for the 5 and 7 liter reactors:

Part Number	Part Description	Quantity Per Assembly
V3MA000031	CLAMPING RING FOR PH-NIPPLE VITON	2
V1S4ARP260	O-RING ID164.70X3.53 SILICONE	2
V1S4ARP124	O-RING ID 31.42X2.62 SILICONE	2
V1S4ARP111	O-RING ID 10.77X2.62 SILICONE	4
V1S4ARP023	O-RING ID 26.70X1.78 SILICONE	2
V1S4085X25	O-RING ID 8.50X2.50 SILICONE	12
V1S4026X19	O-RING ID 2.60X1.90 SILICONE	5
V1S4022X16	O-RING ID 2.20X1.60 SILICONE	2
V1S4020X20	O-RING ID 20.00X2.00 SILICONE	10
V1S400PDR3	RUBBER SEPTUM-SMALL SILICONE GREY	5
V1S4004X10	O-RING ID 4.00X1.00 SILICONE	5
V1S1302007	LIPSEAL FOR ST.ASS.V- 7S FPM BLACK - NON FDA	4
V1S1302001	LIPSEAL FOR ST.ASS.V-10A FPM - FDA COMPLIANT	2
V0W3300006	TWIST LOCK 22.9 - 25.4MM	10
V0W3300005	TWIST LOCK 14.0 - 16.5MM	10
V0W0700001	TUBING CLAMP	6
V0W0530005	REDUCER MALE-MALE 1/16"-3/16"	3
V0W0530004	REDUCER MALE-MALE 1/8"-3/16"	1
V0W0430002	T-CONNECTOR 1/8"-1/8"-1/8"	2
V0W0430001	T-CONNECTOR 3/16"-3/16"-3/16"	1
V0R9900101	ALLEN KEY 1.5 MM	1
V0R9900007	COMP. STORAGE BOX 312x238x51MM, 21 DIVIDERS	1
V0R1451010	CABLE TIE BLACK 92X2.3 MM	25
V0N0000093	KWIK CLAMP PA HOSE OD 9.1-11.0MM	12
V0N0000091	KWIK CLAMP PA HOSE OD 6.5-8.2MM	4
V0N0000090	KWIK CLAMP PA HOSE OD 5.5-6.8MM	6

Z81100AK22 Start-Up Kit II for autoclavable reactors 5 and 7L