CellTumbler documentation

for

CellTumbler cabinet

Model year 2014

Product overview

CellTumbler:

- Is an affordable rocking device supporting the well known cell culture wave bags
- Allow the user to select and use any commercial available wave bags up to 10/20 litre
- Facilitate standardization and interchange ability of most wave bags
- Improved mass transfer with simple turbulence creating mixing system

Either Single or Triple

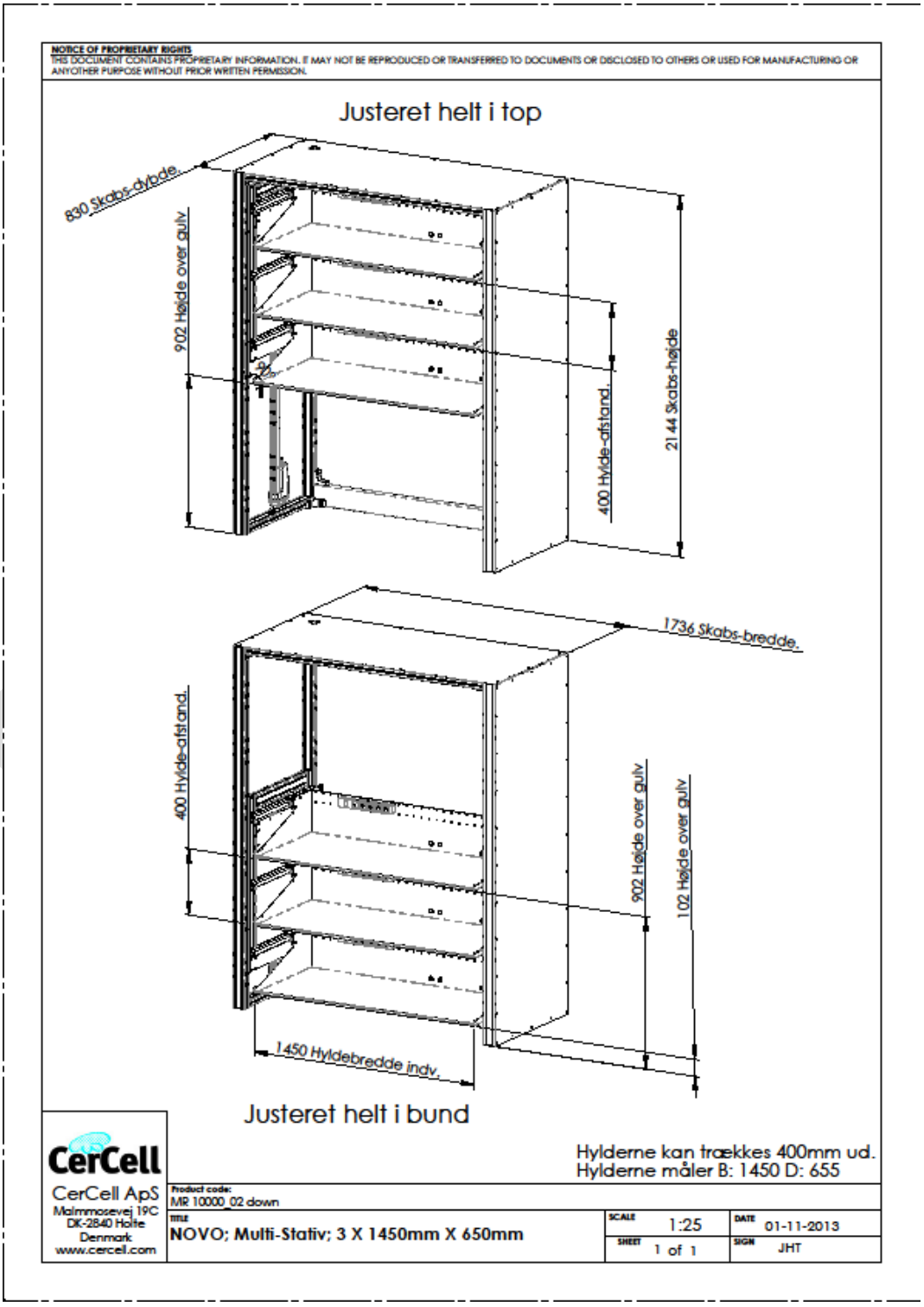
- Three CellTumblers stacked in an ergonomic attractive cabinet on extractable and up/down movable shelves, trays

CellTumbler essential parts are:

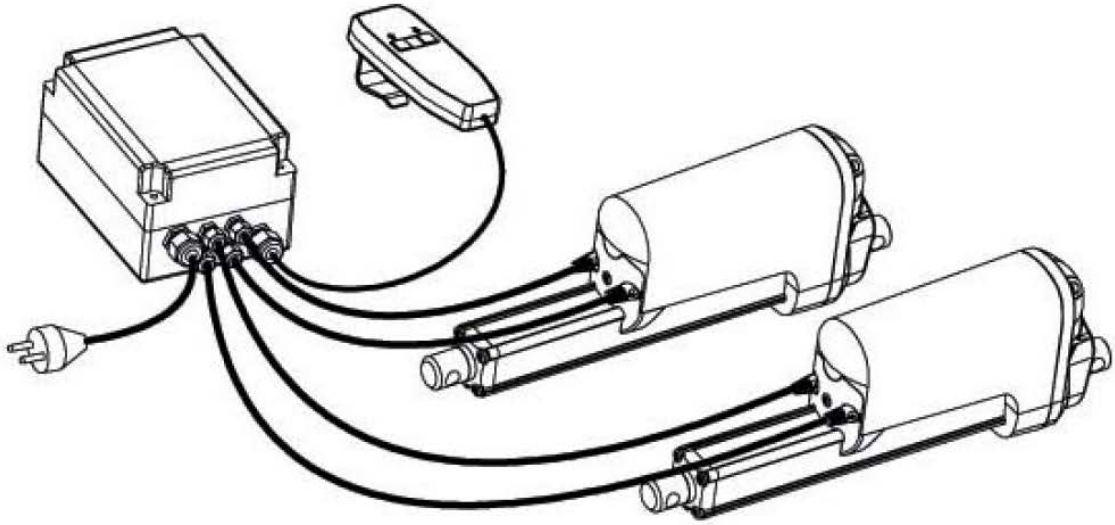
- The platform(s) to which any wave bag may be applied
- The Drive-Unit which handles one or more platforms (depending on the bag size)
- The Heat-Control-Unit with two independent channels for two platforms
- Manual operated Gas-Unit for low cost air/CO_2 control

CellTumbler cabinet (NN#2)

The triple CellTumbler´s are mounted in a 1736 mm wide, 830 mm deep, 2144 mm high cabinet standing on floor towards a wall with counter weight at bottom rear side. Cabinet manufactured from Paletti 40x80 mm aluminium profiles covered with AISI316 stainless sheet on three sides integrating 3 identical 400 mm distance extractable fully welded stainless steel shelf, trays (width 1450 mm) arranged with 400 mm distance to each other. Shelf's manufactured from AISI316 are sliding on ID20 mm ball bearings on X46 hardened stainless steel rails assembled on aluminium profile frames.



Up & Down direction manually control of LA36 actuators via a TP1 unit mounted on the left vertical frame. The three low voltage components are connected to a central actuator drive unit supplied with 230 VAC and mounted inside CellTumbler cabinet. The up/down elevator system are driven by two parallel arranged Linak LA36 electrical actuators operating at 50 mm/sec velocity, 800 mm stroke.



Cabinet shelf
Shelf's manufactured from 3 mm thick AISI316 sheet steel fully welded to a liquid tight construction.
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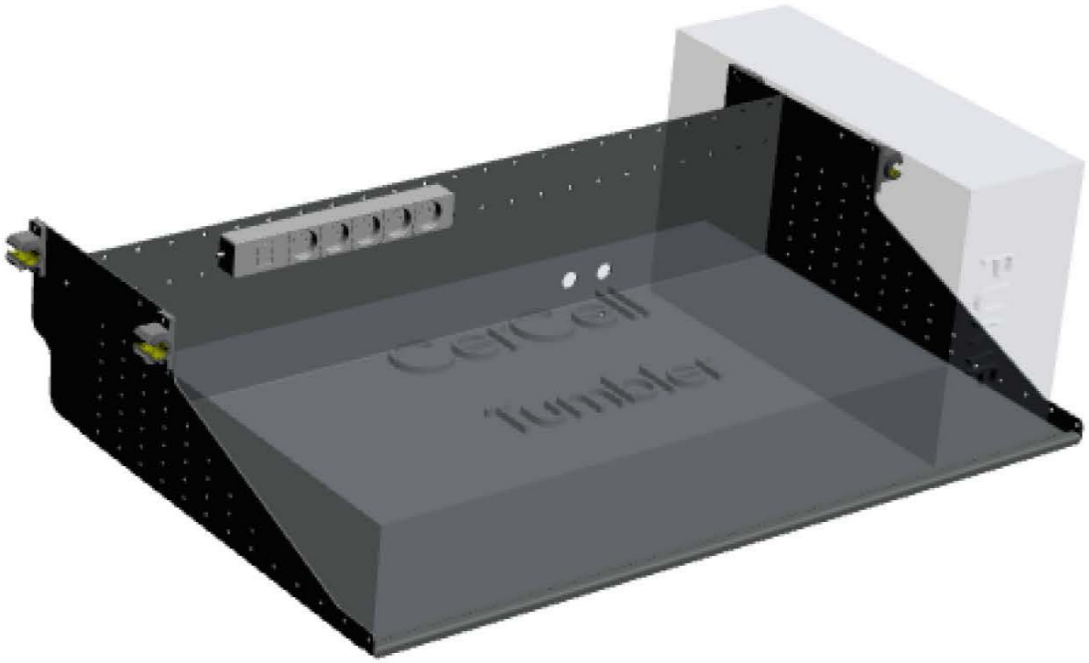


Heat-Control-Unit
Heat-Control-Unit includes two parallel and identical regulating channels in the same box. Sensor input for each of the two channel is Pt-100. ELCO temperature regulator, controller with PI algorithm action and numerous user adjustable features.

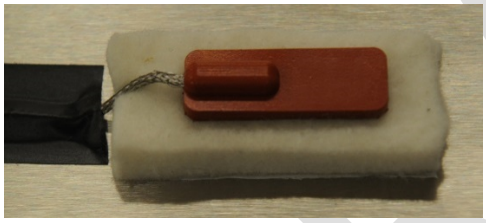
The unit are designed for individual heating element control of two independent platforms.

Connection to 230 VAC only, with standard IEC socket-plug power cables into 2 amp fused and switched IEC Power Inlet Module in IP54 green coat aluminium enclosure with dimension 186x119x79.

The Heat-Control-Unit facilitates on each of two channel:

- One Pt100 sensor input
- One 230 VAC outlet rated at 230 watt for platform heating
- One 18 VAC constant power outlet for one 3 Watt 80x120 mm heating element for OD 50 mm sterile filter heating for 30°C gradient to the environment

Any 3-wire type of Pt100 sensor may be used. Supplied with Silicone patch 40x13 mm with self adhesive foil attached to PET porous non-woven patch. According to DIN EN 60751.

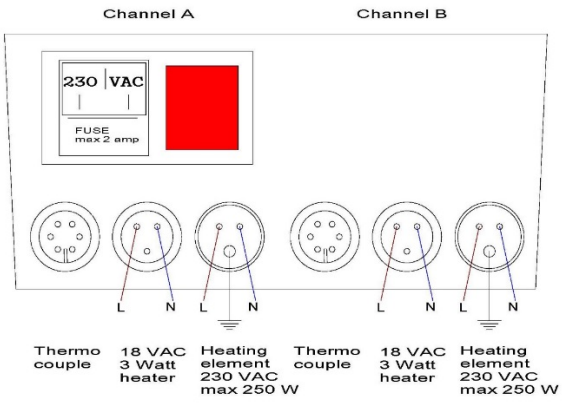


ELCO controller
Advanced controller able to operate within ±0.1°C of accuracy. Units equipped with the ELCO ELK38 regulator is supplied pre-programmed.

At initial system start-up the controller will perform the first auto-tune function in order to measure the thermal mass and response of the system. For optimum accuracy the media (no cells) temperature should be less than 50% of the set point temperature at start up. So if the set point is 36°C the media temperature should be lower than 17.9°C. Allow the system to perform the auto-tuning function on >50% media volume. Collected data is stored to increase accuracy at the following run.

At every system start-up the controller will perform the auto-tune function if the temp diff is larger than 50%.

Your programming is limited to main set-point (see 2.1 in the manual). In general it is recommended to set the main set-point app 0.5-1°C lower than the final target set point and half a day later increase the main set-point to final temperature.

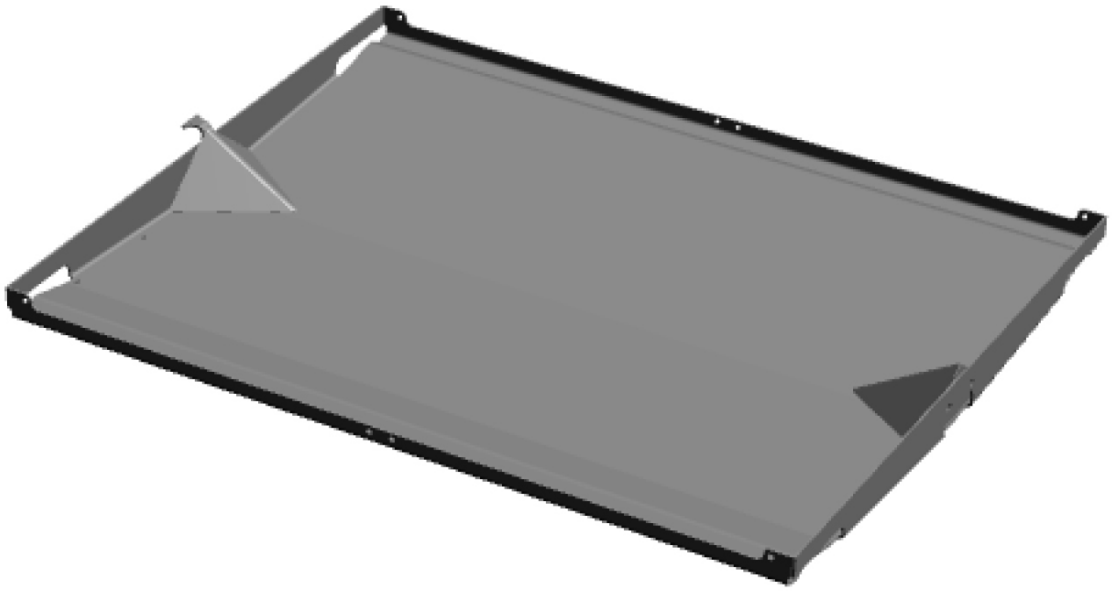


CellTumbler Drive Unit
Brushless 10 watt DC motor with ball bearings, planetary gearbox with ball bearing, support housing with ball bearing for vastly extended service life. Micro processor control motor speed with feed-back for high accuracy and 10-turn potentiometer adjustment of stroke number ranging from 0 to 40 strokes per minute. Stroke width adjustable between 35, 40, 45, 50 mm corresponding 5, 6, 7, 8 degree rocking angle.

Power connection to 230 VAC only with standard IEC socket-plug power cables into 0.5 amp fused and switched IEC Power Inlet Module in black coat IP54 aluminium enclosure with dimension 186x118x80.

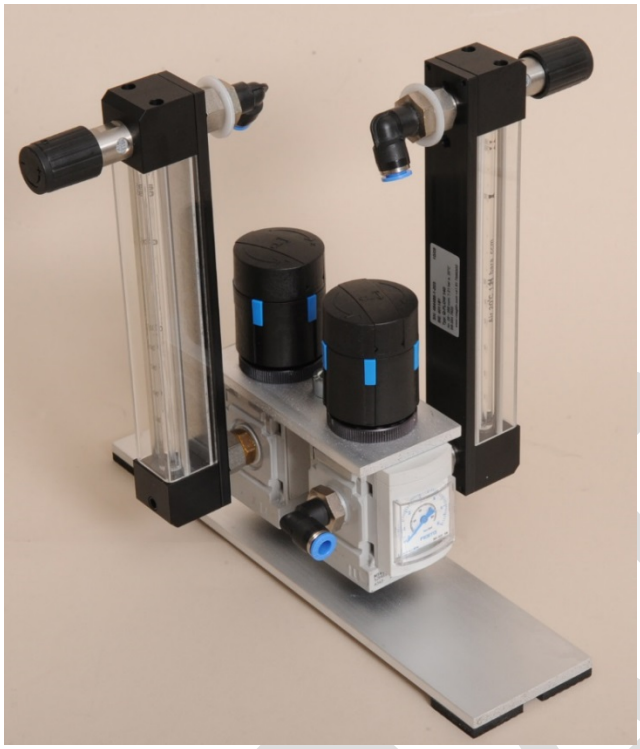
Platforms

Each of the three CellTumbler´s are equipped with a new platform (#300-20) design for increased stiffness manufactured from AISI316 stainless steel. Silicone rubber encapsulated 200 watt, 230 VAC heating elements from FlexElec adhered to the read side with 3M tape to the platforms. The two removable “pyramides” shown on each end of the platform changes the flow pattern inside bag from laminar to turbulent.



Gas-Units

The concept is designed for selection of one or more of the Gas-unit-flow-blocks to be integrated into the aluminium Support-Block or mounted directly on the SS shelfs.



Glass tube rotameter, flowmeter covering a suitable flow range over the 150 mm scale.

- 0-50 ccm / 0.05 ln/m – model 110
- 0-152 ccm / 0.15 ln/m – model 130
- 0-281 ccm / 0.28 ln/m – model 140

- Flow: ccm = cm^3/min
- Flow: ln/m = normal liter per minute
- Rotameter inlet gas pressure: 1.013 mBar adjusted on the Festo pressure regulator
- Air density: 1.293 kg/m^3
- CO_2 density: 1.98 kg/m^3
- Air to CO_2 correction factor: 0.95

Bags

GE Healthcare, Cellbag™

Platform identification	Liquid volume maximum	Bag total fluid volume	Bag dimension width x length	Platform size width x length	Number of bags per platform
300-01	2 x 0.5	2 x 1	550 x 270	550 x 550	4
300-01	1	2	550 x 270	550 x 550	2
300-10-GE	5	10	550 x 330	550 x 340	1
300-10-2-GE	5	10	550 x 330	550 x 685	2
300-20	10	20	550 x 660	550 x 685	1

Sartorius Stedim, CultiBag

Platform identification	Liquid volume maximum	Bag total fluid volume	Bag dimension width x length	Platform size width x length	Number of bags per platform
300-01	1	2	550 x 275	550 x 550	2
300-10-SS	5	10	420 x 500	420 x 500	1
300-10-2-SS	5	10	420 x 500	420 x 1000	2
300-20	10	20	550 x 660	550 x 685	1

Guide-to-use the CellTumbler – ver 4, 2012

Inoculation

Inflate t9he bag with air and 10% CO_2 until bag is rigid, add media and clamp the inlet and exhaust filters. Start rocking the bag at 15 rocks per minute (rpm) and an angle of 7 degrees. Allow the temperature and pH to equilibrate. Add cells to the bag. Cell inoculum should be sufficient to give a starting cell density of at least 5.0 x 10^5 cells/ml. Use a greater cell density if required. Adjust the rpm and angle as described below. Due to the efficient gas exchange that is characteristic of wave bag concept, the pH of bicarbonate buffered media can shift suddenly. During the early stages of the culture keeping the filters clamped helps maintain a steady pH. Refer to the section on pH control for some recommended techniques.

Operation
While the cells are growing monitor cell density, viability and metabolism, adding media when required. If possible add media that has been warmed to operating temperature. Keep the bag rigidly inflated at all times. Make sure that there is good contact between the temperature probe and the culture. This will prevent overheating, which is particularly important if the culture is at a low volume. The oxygen levels of the culture are critically important to successful cell growth and production. Monitor the oxygen levels with a DOPROBE and DO20 amplifier or off-line blood gas analyzer. Adjust the rpm and angle in response to the oxygen demands of the culture.

Rocking speed
The rocking speed is dependent on the culture volume, cell density and bag size. For 1/2, 5/10 and 10/20 bags set at 12 to 20 rpm initially. For very low volumes, 10-20% of the bag working volume, an initial rpm of 12 is sufficient. Increase the rpm to 20 to 25 as more media is added to the culture. When at 100% of bag working volume or at high cell density, the rpm may need to be as high as 25. At 10-20 % of working volume set the rpm at 10. When at maximum volume use an rpm of 22. These are general guidelines only. Monitor the oxygen levels and adjust the rpm and rocking angle as needed.

Rocking angle
For 1/2, 5/10 and 10/20 bags an initial angle of 6 degrees is sufficient. Generally increase the angle as the oxygen demand increases. When the bag is at 100% of working volume, an angle of 7 or 8 degrees may be needed when using 1/2, 5/10 and 10/20 bags. Reduce the rocks/per/minute if excessive foaming is observed. It is important to monitor the oxygen levels of the culture and adjust the rpm as needed.

Aeration rate
Simplicity is the primary theme for CellTumbler. Which is why we recommend a 50 litre / 200 Bar / 10 m^3 bottle with pre-mixed 5% CO_2/Air for gas supply. This simple means also secures correct gas mix at any time for a stable pH level. The bags should be kept rigidly inflated at a maximum of 50-100 mBar overpressure. During bag inflation a flow rate of up to 0.5 ln/m (normal litre per minute) can be used for 5/10 and 10/20 bags. During the initial stages of the cultivation keep the sterile filter (inside the sterile filter heater) outlet arranged to insure sufficient drop collection drained to the bag. Once vigorous growth is observed set the flow rate to 0.05-0.1 ln/m per liter media, such as:

- 0.1 ln/m for the 1/2 bag
- 0.2 ln/m for 5/10 bag
- 0.5 ln/m 10/20 bag

Sterile filter
Its of high importance to mount the 3 Watt constant heater on the outlet sterile filter. This in order to avoid high pressure drop over the sterile filter caused by condensation and foam passing up through the connection hose. Insure the sterile filter is mounted vertical for best possible drain into the bag. Use hangers and nylon strips in order to support the sterile filter heater.

Foaming
High rocking speeds produce foam, which reduces aeration rate and increases the pressure drop over the outlet sterile filter. Blocking the outlet sterile filter will increase bag pressure and potentially damage the bag. The pyramid device increases the media and cell mixing rate dramatically, which allow reduced rocking speed and foam formation.

Temperature
Initial system start-up of the Heat-Control-Unit:
For the ELCO controller – allow the system to operate an hour or so with the auto-tune function on (standard programming) with minimum 50% liquid before adding the cells. Collected PID data from first run will be stored in the controller and used for the next run. Typical operating temperature for 293 cells is 36 to 37°C. See the programming guide.

pH control
The pH control is extremely critical. Due to the high gas transfer capacity of the concept, pH may drift rapidly. Use the following procedure:
1. Initially inflate the bag with 10%CO_2/air. After inflation add media into the bioreactor and close off the inlet and outlet air filters. Allow 1 – 2 hours with rocking at 15 rpm for the pH and temperature to completely equilibrate. Before inoculation check the pH by taking a sample. Adjust if necessary.
2. Inoculate with cells. Leave the inlet and outlet filters closed.
3. Monitor pH, glucose concentration and cell density. In general the CO_2 level could be between 5 and 10% until the cell density reach 0.5 x 10^6 cells/ml. Once the pH and glucose levels start dropping, switch to 5% CO_2/Air mix with continuous airflow through the headspace. This should occur within 24 to 60 hours. Once vigorous cell growth occurs, the media pH will not drift upwards and CO_2 concentration in the sweep gas can be used to control pH.
4. Adjust rock rate to maintain oxygen concentration. Improve liquid turbulent flow adding the pyramid rubber body. Use offline sampling to estimate dissolved oxygen concentration.

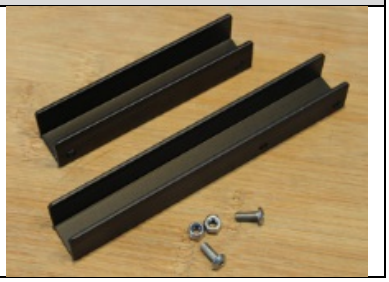
Scaling up
A typical scale up for HEK293 cells in a 10/20 bag is given below. Keep in mind that this is a general guide only.
1. Set rock rate to 12 rpm and the angle at 6-7 degrees. Fill 2 liter of <18°C media into a 10/20 bag. Insure the system operates at desired temperature after >6 hours of auto-tuning from the controller. Add inoculum to give a starting cell count of at least 1 x 10^6 cells/ml.
2. After 2-3 days of cultivation (depending on doubling rate), the cell count should reach about >2 x 10^6 cells/ml. At this stage add 3 liters of fresh pre-heated media to bring the total volume to 5 liters. Increase the rpm to 20.
3. Continue the culture for a few more days until the cells reach >2 x 10^6 cells/ml. Now add final 5 liters of fresh pre-heated media. Increase rpm to 20. Monitor the oxygen levels and pH in the culture carefully.
4. When the desired cell density has been reached add virus at the appropriate MOI.
5. Continue the culture for another day or two until cell viability begins to drop. Determine ahead of time the optimum viability at which to harvest.

For other size bags adjust the volume proportionally. A 5/10 bag would start at 0.5-1 mL and the 1/2 bag at 100 mL.

Tips & Tricks

- Improve mass transfer and productivity at reduced rpm creating media turbulence by adding our “half pyramid” device. Which improves liquid turbulences significantly inside the culture bag – see more under *turbulent flow*.
- Be sure the Pt100 sensor(s) (like channel A) is always in contact with the correct culture bag if the heating element (channel A) plug is connected – otherwise the platform / culture bag will overheat.
- Don’t turn on the Heat-Control-Unit until all the sensors, wires, bags are mounted.
- The CellTumbler is consequently equipped with electric ground, earth from mains inlet to all parts. Be use to use a wall plug with ground. Never use a power cord which is not equipped with ground, earth.
- Each of the 18 VAC (volt alternating current) on the Heating-Control-Unit plugs intended for sterile filter heating element connection will not accept more than one 3 Watt constant power heating element.
- The platform heating elements has an 80 mm not heated spot in the centre. Organise the Pt100 sensor on the insulation pad right in this centre for best possible response.
- The Drive-Unit crank wheel is equipped with a series of threaded M8 holes in different distance from the rotating centre. Use a 5 mm Umbraco / Allan key to adjust the stroke to your need. Stroke of 35, 40, 45, 50 mm are corresponding 5, 6, 7, 8° rocking angle. By default 40 mm / 6 degree.
- Protect the culture bag from draught originating from windows and ventilation systems. Reduce the draught effect by covering the culture bag with bubble-plastic-foil – it is simple and low cost.
- Insure no tubing or wire is rubbing against the bag or other parts. Use nylon strips to secure hoses and wires to the hanger support.
- Take advantage of the hanger support for the sterile filter heating element and insure the filter is vertical for better drainage to the bag.

Most important spare parts

Item #	Description of spare wear parts	
510	Drive U-profile (GE) length 80 mm incl sets of M4x10 screws and locking nuts	
511	Drive U-profile (SS) length 140 mm incl sets of M4x10 screws and locking nuts	

520	Black plastic barrel Ø16ø8 incl bolt	
521	Pedestal feet	
522	Transparent suction cups Ø44 – two mounts onto the T-frame and 2-4 on Gas-Unit - use the Ø3.5x15 stainless steel screws	

Technical and Regulatory documentation for CellTumbler

Platform parts

- Platform, tray – stainless steel AISI316
- Flexible elements – (www.iac-nordic.dk) nickel plated steel support, red natural rubber
- T-frame – Aluminium with surface hard anodised
- Bolts – stainless steel AISI304
- Rubber feet – grey polyurethane
- Rubber feet – transparent vinyl
- Heating element (www.flexelec.com) red silicone heater plates, white silicone cables
- Thermo sensor – (www.tcdirect.co.uk) red silicone patches, nickel plated metal wire flex strengthen cord
- Sterile filter heaters – (www.flexelec.com) red silicone heater plates, white silicone cables
- Plugs (www.bulgin.co.uk) – nylon, glass filed nylon, phenolic, nitrile rubber, contacts: silver plated brass

Drive Unit

- Cabinet (www.hammondmfg.com) – aluminium
- Cabinet paint – AkzoNobel - Interphon Polyester

- IEC Power inlet (www.bulgin.co.uk) – Housing: nylon and glass filed nylon, contact: silver plated brass
- Plugs & sockets (www.bulgin.co.uk) – Housing: nylon, glass filed nylon, phenolic, nitrile rubber, contact: silver plated brass
- Gear motor – (www.intecno.com) – aluminium housing, steel shafts, nylon housing, high viscosity synthetic gearbox grease enclosed by polyacrylic oil seals
- Electronics – circuit boards free from PCB

Heat Control Units

- Cabinet (www.hammondmfg.com) – aluminium
- Cabinet paint - AkzoNobel - Interphon Polyester
- IEC Power inlet (www.bulgin.co.uk) – Housing: nylon and glass filed nylon, contact: silver plated brass
- Controller units – self-extinguishing plastics UL94V0, circuit boards free from PCB
- Plugs & sockets (www.bulgin.co.uk) – Housing: nylon, glass filed nylon, phenolic, nitrile rubber, contact: silver plated brass

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