



## FAQs - 50 questions and responses about HEMOXCell

- 1- What is the effect of the anti-oxidative properties of HEMOXCell to my media?  
The anti-oxidative properties of HEMOXCell destroy the reactive oxygen species (ROS) which damaged the media. The ROS can also deteriorate unsaturated fat compounding the membrane and can kill cells.
- 2- How to define the optimized concentration of HEMOXCell for my process? You have to perform a dose response scale so several test at various concentrations of HEMOXCell must be performed to find the optimum concentration.
- 3- Does HEMOXCell is FDA approved? Not Yet. HEMOXCell is a cell culture additive to better transport oxygen and Hemarina is providing material certificate to qualify HEMOXCell within your process.
- 4- Does HEMOXCell added to media will influence the downstream processing? No
- 5- Does HEMOXCell is animal component free? Yes. Even if the origin of HEMOXCell is hemoglobin from a sea invertebrate animal it's not considered by the FDA as an animal origin.
- 6- What can of documents can I obtain to guarantee the GMP manufacturing process of HEMOXCell as a raw material for my process? Depend on the customer demand we can provide: Sterility, OGM free, mycoplasma free, endotoxin free and DNA free certificates.
- 7- What is the lifetime of HEMOXCell when stored properly in a deep freezer? Lifetime of HEMOXCell is 12 months (from date of delivery) and stored at -80°C. Storage at a higher temperature (e.g freezer at -45°C or -30°C) can affect the properties of HEMOXCell.
- 8- What are the ideal temperature conditions for using HEMOXCell during a cell culture process? Between 4 and 37°C
- 9- Can I use HEMOXCell without a sterile barrier into a CO2 incubator? If production support is maintained sterile then it's yes. If not the culture could be potentially contaminated.
- 10- During a cell culture process can I use my oxygen set-point to control feeding of HEMOXCell? This type of culture condition has not been communicated yet. This is possible but it will need precise oxygen measurement and set-up adjustments of the feeding (on/off or PID set-up of the pump rate).
- 11- Finally is it possible to adjust my feed(s) to the control of oxygen or another related event? This type of culture control of oxygen has not been communicated yet. This is a complex set-up that will need time and several adjustments before reaching an optimized control loop.
- 12- What is happening to HEMOXCell when oxygen is released to cells? HEMOXCell return to his natural form but without oxygen.
- 13- How could you guarantee the efficiency of HEMOXCell from a manufacturing batch to another? Several test of efficiency are generated with CHO cells with each manufacturing batch to release commercially a batch. If the test failed then batch is not released.



- 14- What could happen to HEMOXCell during an unexpected thermal shock? HEMOXCell can be damaged and its functionality reduced. It's important to follow the instruction manual to avoid such temperature issue.
- 15- When removed from the deep freezer how Hemoxcell should be prepared and stored? HEMOXCell should be defreeze at room temperature and use directly after the complete defreezing phase. See instruction manual for further information about the defreezing process.
- 16- If I don't need to use the quantity ordered can I reuse the quantity left? Yes you can freeze/defreeze 3 times. But this could affect the properties of Hemoxcell so we don't advice such practice.
- 17- During a batch or a fed-batch process HEMOXCell is added gradually? Separately? Mixed with all media before inoculation? HEMOXCell® is added at the beginning of the culture directly to the media before inoculation during a batch process. For a fed- batch process, HEMOXCell must be added gradually to the media feeding and cell density. There is 2 ways to manage a fed-batch process by adding separately and gradually HEMOXCell to the bioreactor or pre-mixed with media (stored at +4°C).
- 18- Preparation of HEMOXCell is strictly operated with the buffer from Hemarina or can I use another buffer? Strictly used with the buffer from Hemarina. If other buffer is used then we will not guarantee the performances of HEMOXCell.
- 19- Can I use HEMOXCell with an acoustic perfusion device or ATF? This type of culture condition has not been communicated yet
- 20- HEMOXCell can be reloaded with Oxygen? If so how? Yes by creating an oxygen gradient with an environment where the partial pressure would be lower than the P50 of HEMOXCell®: bubbling with O<sub>2</sub> or agitating for example.
- 21- Can I use HEMOXCell to store my cells? If so what are the conditions of storage? Yes but has to be tested with the cell line considered and it's also depending on the media and storage conditions. Usually a difference is observed with cells stored with HEMOXCell.
- 22- Does Hemoxcell is accelerating the accumulation of lactate/ammonium, depletion of glutamine or secretion of any material able to affect my process? This type of information has not been communicated yet.
- 23- How my carbon source or other media components should be adjusted when using HEMOXCell? This type of information has not been communicated yet
- 24- Hemoxcell can be used with any type of cells? Yes, many tests have been performed with CHO, SP2/0, MSCs and other test with other cell lines are in progress.
- 25- Can I use HEMOXCell with an adherent process with Cytodex? Yes. A carrier as Cytodex3 could be used as long as the cells are in contact with media.
- 26- Can I use HEMOXCell when my cells are attached to a scaffold? Yes. Using a scaffold in 2d or 3D is possible with HEMOXCell as long as the cells are in contact with media.
- 27- What is the concentration range and cell density obtained with HEMOXCell? There is no precise set-up and several tests should be performed to define the optimum concentration of HEMOXCell. For example, after several batch cultures with same conditions (media and cell line).



The optimum concentration was for this project considered at a concentration of 0.025mg/L with a specific bioreactor. With an inoculum of 50000cells/ml, after 6 days we obtained a VCD of  $14 \times 10^6$  cells/ml. This is an example that is only use as an indication for your specific process: cell line, media, cell density targeted, bioreactor design, etc.

- 28- Does Hemoxcell can affect the viability of my cells during culture? No. Actually HEMOXCell is helping cells to divide and mature in a better environment so a better viability is usually observed
- 29- Can I use HEMOXCell with any type of Bioreactor? Yes. From MTP to large volume of culture HEMOXCell can be use. The volumetric productivity is enhanced depending of the environment. This is demonstrated with MTP, T-flask, roller bottle, spinner etc., and the volumetric productivity is enhanced. For example the filling of a shake flask is higher, speed of the shaker reduced and the overall productivity is enhanced because the orbital surface is not affecting the transfer of oxygen anymore. This is a very interesting advantage using HEMOXCell.
- 30- Can I order HEMOXCell in bulk quantity? Yes. Such project could be quoted by our sales team based on the total quantity of HEMOXCell ordered as a batch. Packaging could be also modified for such demand.
- 31- What is the origin of HEMOXCell? HEMOXCell is extracted from a marine worm (classified as invertebrate marine source) called "Nereis virens". Farming is fully controlled by Hemarina (owner of the farm) and when the adult phase is achieved (1 year) then the hemoglobin (commercially named HEMOXCell) is extracted and purified following a proprietary patented method. From cell banking to the commercial product, Hemarina is controlling the whole process.
- 32- What is the timing and transportation condition of HEMOXCell? Timing: 3 days door to door; Transport condition:  $-80^{\circ}\text{C}$  under dry ice. Shipment is released each Tuesday from the factory.
- 33- Why can't I use HEMOXCell for a fermentation process? The quantity of HEMOXCell will be too high because the respiratory quotient of a bacteria or yeast is much higher than mammalian cells.
- 34- Can I use Hemoxcell and continue to use sparger/bubbling with air/O<sub>2</sub> my cells? If HEMOXCell is enough to enhance the volumetric productivity of a considered process of culture then there is no reason to supplement with an addition of air or oxygen. Mixing both source of oxygen could generate an oxygen overboost and higher cell mortality due to bubbles. Making such experiment to achieve for example a faster growth rate could be possible. Such oxygen supplementation must be optimized to reach ideal conditions if effective. Adding HEMOXCell has also the advantage to reduce the shear stress of an agitation because mixing and transfer of oxygen is released differently. Usually when using HEMOXCell the filling of the bioreactor, the type of agitation and speed could be also reduced (limited) or changed to reduce even more the shear stress of cells.
- 35- Does Hemoxcell is affected when CO<sub>2</sub> is used to adjust pH? HEMOXCell has a low affinity with CO<sub>2</sub> so it could affect the efficiency of Hemoxcell when CO<sub>2</sub> is sparged. Normal conditions (stable pH) of a culture should not affect much the use of Hemoxcell.



- 36- Can I reduce the speed of agitation and eventually change impeller when using HEMOXCell? **Yes**, this is an important property of using HEMOXCell. Various experiments will be needed to demonstrate such advantages including reduced agitation speed gradually (shaker, bioreactor), no more pressurized bags, different impellers (smoother mixing) etc.
- 37- If my bioreactor is pressurized, is it affecting the properties of Hemoxcell? **No**
- 38- Hemoxcell can be used with any type of culture media? **Yes**
- 39- When using a hollow fiber or filtration device couple to my bioreactor is it removing HEMOXCell with permeate? What cut-off is advised? **Molecular weight of HEMOXCell is 3.6 MDa and its dimensions are 25x17nm. HEMOXCell® will be disposed with the permeate is the cut-off of the membrane is higher than this dimension.**
- 40- Is there any side effect for my cells if I'm adding media additives or growth factors when using HEMOXCell? **No**
- 41- Is there a safe method to dissolved or removed HEMOXCell from media? **Could be for example a separation process or a method precipitating HEMOXCell at the isoelectric point of the molecule if the pH is compatible with the process. Contact us if you have any questions concerning the way HEMOXCell could be removed from the media.**
- 42- My cells are cultivated at a temperature higher than 37C, is it affecting the properties of HEMOXCell? **Such culture conditions has not been tested yet or communicated**
- 43- Is it possible to reload oxygen to HEMOXCell? **Yes**
- 44- Can we use Hemoxcell for constructing of tissues for R&D purposes? **Yes**
- 45- How can I find out the efficiency of a lot of Hemoxcell prior a culture? **There is no particular method validated at customer site so contact us if you have any question apart the certificate provided by Hemarina.**
- 46- Is it possible to have Hemarina offering quality control of an existing lot? **Yes. Samples can be sent back to our QC lab for verification.**
- 47- Is it possible to track a lot history? If so for what period of time? What kind of document can I obtain from Hemarina? **Yes all records are maintained on file for a 10 years period.**
- 48- Is there IQ/OQ documents available at Hemarina? **Such protocol and service is available on demand.**
- 49- Is it possible to have Hemarina quality personnel available during a PQ phase? **We highly recommend it to validate the way Hemoxcell is used for the qualification of the process.**
- 50- I need more information, document, who should I ask? **Please contact your distributor at [customerservice@lifetechindia.com](mailto:customerservice@lifetechindia.com)**



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