



Bioprocessing Containers

VueLife® Cell Culture Bags

KryoSure® Cryopreservation Bags

VueLife® Cell Culture Bags
for Adherent Cell Lines

FAQ

Content

I. TECHNICAL AND MATERIAL INFORMATION ABOUT BAGS FROM CELLGENIX

1. What is the density of FEP as used in VueLife® bags?	4
2. What is the modulus of elasticity of the material in VueLife® bags?	4
3. What is the coefficient of thermal expansion of the material in VueLife® bags?	4
4. What is the tensile strength of the material inVueLife® bags?	4
5. How thick is the FEP-film of the VueLife® bags?	4
6. What are the inert properties of the VueLife® bags?	4
7. What are the permeability properties of the VueLife® bags?	4
8. What is the temperature range of the VueLife® bags?	4
9. What is the surface texture of the film under an electron microscope?	4
10. What is the expiration date of VueLife® bags?	4
11. What are physiologic temperatures?	5
12. Will FEP react with chemicals outside these temperature ranges?	5
13. What is the best way to connect VueLife® bags?	5
14. Most of your VueLife® bags have Luer fittings. Can you put a larger diameter fitting on your VueLife® containers?....	5
15. How long would it take to produce a special customer bag?.....	5
16. The culture container line now has something called a Needleless Injection Site. What is that device?.....	5
17. What is the outside and inner diameter of standard PVC tubing?.....	5

II. USE OF VUELIFE® BAGS

1. Could your VueLife® containers be used to culture Islet cells?	6
2. Can VueLife® FEP bags be used with cells which require activation of a binding site such as an FC receptor?	6
3. Why go to all that trouble when I can use a T-flask?	6
4. How can I see if cells are growing in the VueLife® bags?	6
5. What is the best way to harvest cells from the VueLife® bag?	6
6. What size of VueLife® bags are used for the culture of dendritic cells?	7
7. About cell culturing in VueLife® bags.	7
8. How many cells can grow in a VueLife® bag?	7
9. Is it possible to write directly on the outside film of the VueLife® bags?	8
10. Would it be possible to produce VueLife® bags with collagen inner covering or with other substances covering?	8

III CRYOPRESERVATION WITH KRYOSURE® BAGS

1. Can VueLife® bags be frozen safely at –80 °C?.....	9
2. What is the difference between VueLife® bags and KryoSure® bags?	9
3. When do you recommend using overwraps?	9
4. Are there overwraps for VueLife® and KryoSure® bags from CellGenix?	9
5. Is it possible to use a clamp instead of sealing the bag or overwrap?	9
6. What items are necessary for cryopreservation of cells in liquid nitrogen?	9

Content

7. What heat sealers are available and what temperature is necessary to make the seal on FEP bags?	9
8. How can inflation be avoided?	10
9. Would it be better to set the freezer to -60 °C to avoid the gas thawing problem?	10
10. Do you have special bags for freezing such as in nitrogen?	10
11. Could KryoSure® bags break?	10
12. Why don't KryoSure® bags break when they are removed from the freezer?	10
13. Will KryoSure® FEP bags break in immersion liquid nitrogen storage?	10
14. Is it necessary to get all the air out of the fluid stored in the KryoSure® bag when placing it into liquid nitrogen?.....	11
15. What types of over wraps (KryoSure® FEP pouch over wrap versus Kapton/FEP peel pouch KP-0512) should be used to protect KryoSure® bags?	11
16. Can liquid Nitrogen go through the FEP film of KryoSure® bags once the bag is immersed into liquid nitrogen?.....	11

IV. USE OF VUELIFE® ADHERENT CELL BAGS

1. What is a VueLife® AC bag and what is it for?	12
2. Are the adherent properties strong enough to collect monocytes from buffy coats?	12
3. Is it possible to separate Monocytes from mononuclear cells by using VueLife® AC bags in combination with DC-medium in advance?	12
4. Instructions for use of VueLife® AC bags having an activated surface	12
5. How much retronectin should be used to coat the inside VueLife® AC bags?	12
6. Is it possible to make silicone rubber bags?	13
7. Removing adherent cells from VueLife® AC bags	13
8. What types of cells have adherence in VueLife® AC bags?	13
9. How many cells can grow in an VueLife® AC bag?	13

V. VALIDATION OF VUELIFE® / VUELIFE® AC BAGS

1. Have your bags ever been approved for anything?	14
2. Are your bags approved for working with a human cell line that maybe used in immunotherapy?	14
3. Is there a validation procedure for KryoSure® bags?	14
4. Why do bags need regulatory approval?	14
5. Can I use VueLife® / VueLife® AC / KryoSure® FEP bags in my research?	14

VI. GENERAL INFORMATION ABOUT VUELIFE®/ VUELIFE® AC / KRYOSURE® BAGS

1. What is the difference between FEP and Polyolefin bags?	15
2. What is the advantage of using FEP instead of EVA (ethyl vinyl acetate)?	15
3. Which Bags are the best to do transduction / gene transfer of cells?	15
4. How could VueLife® bags be centrifuged?	15
5. DMSO & VueLife® bags	16

Product List Bioprocessing Containers	17
---	----

I. TECHNICAL AND MATERIAL INFORMATION ABOUT BAGS FROM CELLGENIX

1. What is the density of FEP as used in VueLife® bags?

2.15

2. What is the modulus of elasticity of the material in VueLife® bags?

0.05 x 104 MPa (0.07 x 10⁶ psi)

3. What is the coefficient of thermal expansion of the material in VueLife® bags?

170 x 10⁻⁶ m/m/K

4. What is the tensile strength of the material in VueLife® bags?

2700 psi.

5. How thick is the FEP-film of the VueLife® bags?

0.005 inches (= 0.127 mm)

6. What are the inert properties of the VueLife® bags?

FEP does not adsorb or bind any known chemical or biological. It contains no plasticizers, leachable nor any extractable. Simply put, cells do not stick to the container and nothing from the container adversely affects the cells.

Biologically inert

The bag does not contribute to or detract from biological processes

Chemically inert

Nonreactive, noncatalytic and insoluble

Optically inert

Optically transparent even to U.V. light

Immunological inert

Is non-antigenic to growing cells

7. What are the permeability properties of the VueLife® bags?

The high gas and low water or water vapour permeability of the bags make them uniquely suited for use in CO₂ incubators.

High Permeability to CO₂ and O₂ (the highest of any plastic).

Cultures can be incubated directly in bags.

Low Permeability to H₂O or H₂O vapour (the lowest of any plastic).

Humidified incubators are not required as there is virtually no water loss due to evaporation.

8. What is the temperature range of the VueLife® bags?

FEP maintains its pliability and integrity under a wide temperature range regardless if it is stored in liquid nitrogen or at the other extreme of autoclaving. Glass Transition Temperature < -208 °C FEP bags can be stored in liquid nitrogen (-196 °C). Melting Temperature > 300 °C FEP bags and contents can be autoclaved (121 °C) for sterilization and safe disposal

9. What is the surface texture of the film under an electron microscope?

– not an atomic texture

– not crystalline

The texture depends on the temperature conditions. The common containers are very smooth, so neither cells nor proteins stick on the VueLife® bags. The bags of the AC® Line (for adherent cells) have a changed texture (charged) and so cells and proteins could stick (please see chapter IV).

10. What is the expiration date of VueLife® bags?

VueLife® bags have a shelf life of three years from date of manufacture unless otherwise indicated by an expiration date on the label. The manufacture date is encoded in the six-digit lot number. The lot number is punched into the fringe material of most of VueLife® bags. The lot number is on the packaging material of small or closely trimmed containers. The first three digits (on the left) of the lot number is the Julian Date

(day of the year – a number between 1 and 365). The fourth digit (from the left) is the year (2006 will have a „6“). The two digits on the right represent the number of lots made that day (starting with zero for the first lot number). For example: lot number 123400 will indicate the container was manufactured on the 123rd day of the year 2004 (May 3, 2004). The expiration date for this bag was the 123rd day of the year 2007 (May 3, 2007).

11. What are physiologic temperatures?

Any temperature at which life can exist is a physiologic temperature. Certainly -196°C to $+100^{\circ}\text{C}$ are included as physiologic. Within these temperatures, FEP will not react with any chemical.

12. Will FEP react with chemicals outside these temperature ranges?

Yes. For example, FEP will react with molten sodium if the FEP is at a temperature above 300°C . And FEP will react slowly with a few halogenated compounds when the temperature is above 150°C . Below 150°C , FEP is inert to all biologics and chemicals.

13. What is the best way to connect VueLife® bags?

The best way to connect VueLife® bags is by use of sterile docking. CellGenix recommend use of the Terumo Sterile Docking Device (SCD) which connects to the pre-attached tubing of the standard CellGenix VueLife® bags.

14. Most of your VueLife® bags have Luer fittings.

Can you put a larger diameter fitting on your VueLife® containers?

Yes, we can put FEP tubing with 3/8 inch (9.5 mm) inside diameter. This terminates in tubing that can accept barbed fittings. This fitting is not available on all bags, and requires a special set up charge.

15. How long would it take to produce a special customer bag?

Small changes at a standard bag (e.g. size).
Time: Thirty days minimum.
Please ask CellGenix for detail and formal quotation.

Significant / substantial changes: new master file and more paper work is necessary
Time: 1–2 months
Please ask CellGenix for detail and formal quotation.

16. The culture container line now has something called a Needleless Injection Site. What is that device?

It can also be referred to as a FLV (Female Luer Valve). It is a small device about 5 x 10mm that is incorporated into all VueLife® cell culture containers and connects to any male Luer fitting. The fluid path is closed. When a syringe is attached to the FLV, the valve opens and the syringe can inject or remove fluid. When the syringe is removed, the valve to the fluid path closes again. This is used to permit sampling or connecting to bags. In addition to syringes, Intra Venous (IV) tubing connectors with male Luer fittings and taper fit needle adapters also work with FLV.

17. What is the outside and inner diameter of standard PVC tubing?

Standard size is 4 mm outside diameter and 3.5 mm inner diameter.

II. USE OF VUELIFE® BAGS

1. Could your VueLife® containers be used to culture Islet cells?

Yes. There are several bags available for digesting, washing and culturing of Islet cells.

Digesting of organs is done with the CellGenix 3PF-0270 bag (only on request) that is 5.75" x 11.5" with one side entry to accept a double-fist sized organ. The side entry can be closed with a mechanical clamp (VueLife® 1C-0022 order number 3702). A port at the top and bottom of the container permit perfusion of digesting media. The ports have female Luer fittings centred at the apex of cones to permit harvesting of any free floating cells. The bag is transparent and is completely inert. The containers remain strong and pliable at freezer temperatures.

The VueLife® 4P-2410 sedimentation bag (only on request) is approx. 17" x 20" and has four ports, one at each corner. The VueLife® bag is suspended from its metal reinforcing rod with one port at the top. Cells and washing solution enter at one side at a low flow rate. Input flow can be by gravity from the 3PF-0270 VueLife® bag or can be input by pumping. Cells settle into the bottom corner of the bag. Wash fluid is allowed to flow in the bottom corner port at a higher flow rate, this keeps the cells suspended while they are being washed. Wash fluid exits the top of the container. During the wash procedure about 4 litres of fluid is contained inside the bag; less if the container is supported. Mixing is gentle, so small cell aggregates are not disrupted. At the conclusion of washing, the bag is permitted to go flat as the supernatant is taken off the top. Control is by looking at the VueLife® container and adjusting roller clamps on the outlet tubing that works as a siphon. Because the bag is transparent and observed by eye, control is manual. This is a low-tech process that does not require a computer, but the researcher must supply a suitable rack to suspend the various bags at different heights. Different heights generate the desired flow rates. After the supernatant is siphoned off, culture media is added either from the bottom or the side. Cells can be cultured in the same VueLife® container. The unused port can be reserved for aseptic entry at a future time. Centrifugation is avoided. Culture of Islet cells can be done in the wash bag that avoids the trauma of transfer. But this bag is large and may not fit incubators therefore culture can be done in VueLife® 2PF-0290 (approximately 5" x 10") VueLife® container which permits 200 to 800 ml

of culture media per VueLife® container. During culture these bags should be supported on wire racks of the type used to cool cookies and cakes. Wire racks permit respiration from the bottom of the container. Media is changed by hanging the container for 10–20 minutes which permits the cells to settle followed by siphoning the supernatant off using the top port. Cells can be visualized through the bag with a jeweller's loupe.

2. Can VueLife® FEP bags be used with cells that require activation of a binding site such as an FC receptor?

The standard VueLife® FEP bag will not activate an FC receptor. An FEP bag will not bind or react with any known chemical at physiologic temperatures. The exception would be the VueLife® Adherent Cell (AC) culture bags.

3. Why go to all that trouble when I can use a T-flask?

The VueLife® bags are closed systems that cannot spill or become contaminated. The bags also breathe through the walls so there is no requirement to leave the cap loose. The incubator need not be humidified because there is virtually no water lost from VueLife® FEP bags. (Water is lost from silicone containers.) Fluids and cells can be removed from bags after sterile connection to a transfer tubing. With T-flasks, it is necessary to remove the cap, permitting contamination. VueLife® bag transfers can be done anywhere. T-flask transfers can only be done in a laminar flow bio cabinet, but more importantly, the surface of the VueLife® bag does not provide any adherent surface for cytokines or elaborated proteins, whose loss might hinder the culture, to become bound.

4. How can I see if cells are growing in the VueLife® bags?

Place the bag containing the culture on the stage of an inverted microscope. The cells will be on the bottom of the bag. The microscope will look upward at the bottom of the bag. The bag is completely transparent. You can see the cells. It is common practice to dilute the culture once the cells have grown enough so that the cells touch each other. This is called „growing to confluence“. At that time, an equal amount of new media is added to the culture.

5. What is the best way to harvest cells from the VueLife® bag?

The „supernatant“ fluid that is above the cells is usually removed first. Suspend the bag with the ports of the container at the top as follows:

Attach a tube to one of the ports and connect the other end of the tubing to a supernatant collection container. Let the cells settle to the bottom of the bag for about one to 4 hours. Then without shaking the container, drain the supernatant fluid into the super - natant bag by opening the tube. If necessary squeeze the VueLife® bag slightly. Watch through the container to see that cells are not lost into the supernatant container. When most of the supernatant fluid (about 90 %) has been transferred to the supernatant VueLife® container, close that tube. Resuspend the cells by gently shaking the cell bag. Attach a new tube to the cell container and transfer the cells out with that tube.

6. What size VueLife® bags are used for the culture of dendritic cells?

Dendritic cell cultures are usually maintained in VueLife® bags that hold between 72 ml and 290 ml of culture media. That is CellGenix catalogue number: 72-C (order number 3301), and 72-AC (order number 3331), 197-C (order number 3303) and 197-AC (order number 3333), and 290-C (order number 3304) and 297-AC (order number 3334).

7. About culturing in VueLife® bags:

WHY CULTURE: Cells are generally cultured for one of three reasons:

1. to expand the number of cells as in cord blood, T-cells and NK-cells
2. to produce a soluble substance for harvest.
3. to mature cells such as MNCs to DCs

EXPANSION: For cell expansion, it is desirable at the end of the culture period to be able to harvest the cells. Generally, the supernatant is not as important. It is important that cells do not adhere to the bag.

PRODUCTION: When using cells to produce something, it is necessary to harvest the supernatant periodically. Harvesting the cells is not as critical. When producing a biological, it is essential that the critical biological is

not lost to the bag; either adsorbed onto the surface or absorbed into the bag.

BAGS: In both cases culturing in bags permits the contents to be placed into a centrifuge for separation. It is virtually essential to be able to see through the bag to perform this separation in an efficient manner. It is possible to lightly centrifuge the bags if they are properly supported.

CELLS LINES: The culture process is different for each cell line. Some cell lines must adhere in order to reproduce, and others like to float in suspension. Either method can produce expansion or production. The mechanics of culture will depend on the cell line being grown, as well as the purpose for which they are being grown. All types can be grown in bags.

8. How many cells can grow in a VueLife® bag?

Start with 1 x 10⁶ per ml. Here is why:

IF TOO FEW: If you start with too low a concentration, there may not be enough cell to cell interaction to support the culture at all and the cells may die. This is not from loneliness; it is from lack of cytokines.

IF JUST ENOUGH: If you start with just enough, it will take time for the concentration to reach a critical mass for exponential growth or for maximal production.

IF TOO MANY: If you start with too many, exponential growth will not occur, maximal production will not occur.

WHAT MAKES THE DIFFERENCE: In the VueLife® culture container respiration is through the walls of the containers, so it is not necessary to agitate, mix, rock, or rotate the containers. It is possible that some cultures may benefit from rocking; this is to be determined by the clinical technician. The cells settle to the bottom of the bag. This means that most respiration will occur through the bottom of the container, therefore, put the bag on a screen, not on a solid flat surface. One good option is to use our CellGenix culture cassette (order number 3754). Do not fill more than one centimetre thick, because the additional media above the cells will not improve nutrient or gas diffusion, but of course this depends on the cell type and the individuals own protocol.

II. USE OF VUELIFE® BAGS

CELL CONCENTRATION: Since there is no agitation, the cells will settle into a layer on the bottom. Increasing cell concentration will cause the layer to be thicker. Increasing the thickness of the media fill will also cause the layer to be thicker. The thickness can be expressed as $1X10^6/cm^2$

VALIDATING: Start with one million per millilitre. Your particular cell line may require more or less, you will know in short order.

ADHERENT CELLS: Cells cannot adhere to FEP. It will be necessary to provide them with a surface to stick to. For adherent cells please have a look at the Chapter IV „Use of VueLife® AC bags“.

SPLITTING AND MEDIA CHANGE: With CellGenix bags, cells can be pelleted by centrifuging the container (with proper support) and expressing the supernatant out with a plasma extractor. The culture can be split into aliquots by mixing the bag and removing a given volume or weight to another container.

DISPOSAL: Final disposal of cultures can be safely done by placing the entire CellGenix bag into the autoclave and running a standard cycle. The contents will be contained and sterile. The KryoSure® Line are special bags for freezing in mechanical, vapour phase, or liquid nitrogen. While all VueLife® bags can be frozen in Liquid Nitrogen, some fittings are not recommended. The new line of F Series bags may be used in LN if the use of a bar sealer to close off and remove the inlet port as recommended is followed. Be sure to discuss this requirement when you need this property. Please have a look at Chapter III „Cryopreservation“.

9. Is it possible to write directly on the outside film of the VueLife® bags?

No, this is not possible, because the surface will not accept the ink. There are pens that write on FEP, but you could rub the ink off with your fingers, so there is no permanent way to write on the material at this time. There are some labels that will stick to the FEP material, even in vapour phase freezing, but that is still not a desirable approach, and there is the chance the label could come off. A label on a culture bag is counterproductive as it prevents need gas transpiration. Also, you could not be sure that no chemicals got through the bag to contaminate your media or cells.

VueLife® bags are produced with a hanger hole for a tag. If a label pocket is desired the user could use the KryoSure® products for cell culture. Those bags also have the possibility of creating three numbered segments that are unique to the one particular bag that the tubing came from.

10. Would it be possible to produce VueLife® bags with collagen inner covering or with other substances covering?

No, this is not possible, because the collagen would be oxidized while shipped to the customer. In general no proteins stick on the standard VueLife® bags, but there is the possibility to use the VueLife® AC bags for adherent cells. Just follow the available instructions to cover the inner side with Retronectin or Fibronectin and then you could cover the inner side with collagen or other proteins.

III. CRYOPRESERVATION WITH KRYOSURE® BAGS

1. Can VueLife® bags be frozen safely at –80 °C?

Not really. One would have to bar seal the Needleless Injection Site area closed and protect the PVC inlet tubing from shock while frozen. The rest of the bag will be okay. You should use the special KryoSure® bags CellGenix is offering. They have a special label pocket for the PVC tubing segments and allow for bar sealing off the inlet port.

2. What is the difference between VueLife® bags and KryoSure® bags?

KryoSure® bags have five principle differences: First, both the VueLife® and KryoSure® bags have rounded corners, but the rounded corners in the KryoSure® serve a specific purpose. KryoSure® bags are designed with rounded inside corners to avoid creation of sharp ice points that can spear through the bag.

Secondly, their ports are enclosed within the envelope of the bag to prevent nitrogen or anything else from contacting the ports. Contact can bring contamination and cause freeze failure.

Thirdly, all fittings on KryoSure® bags can withstand liquid nitrogen temperatures safely. The spike ports are made of FEP, and are covered individually by the bag envelope.

Fourth, the entrance port is designed to be bar sealed and the entire filling port is removed before freezing.

Fifth, the KryoSure® bags have 3 uniquely identifiable numbered segments that correspond only to the single individual bag.

In summary, VueLife® bags are designed for cell culture and for storage of materials. KryoSure® bags are designed for freezing at temperatures as low as –196 °C.

3. When do you recommend using over wraps?

We recommend using over wraps when it is important to protect the sample from any contamination that may have infected the freezer. Any microbe that is in a freezer may remain cryopreserved for a very long

time, and may be infective when thawed. This includes microbes that have escaped from bags and vials that have broken or had their caps popped open in the freezer. We recommend over wraps when it is necessary to open the sample in a sterile or controlled area.

III. Cryopreservation with KryoSure® bags Over wraps permit the sample to be dropped into a sterile field. We recommend over wraps when a second line of containment is required if the first bag should fail. The over wrap then protects the freezer as well as the sample.

4. Are there over wraps for VueLife® and KryoSure® bags available from CellGenix?

Yes, over wraps are available in FEP and can be used for any bag.

5. Is it possible to use a clamp instead of sealing the bag or over wrap?

Yes, it is possible. The downside is that FEP plastic like most plastic will compress. This occurs even in low temperatures. The result is that after time, the mechanical clamp could possibly become loose and the seal cannot be guaranteed. When mechanical clamps are removed after being sealed for several days, they appear to be loose even if the sample has not been compromised.

6. What items are necessary for cryopreservation of cells in liquid nitrogen?

You need a KryoSure® bag, an FEP over wrap available from CellGenix, and a sealer to seal both the KryoSure® and the over wrap. Other equipment such as a controlled rate freezer, sterile docking device and other clamps maybe needed.

7. What heat sealers are available and what temperature is necessary to make the seal on FEP bags?

For US market:

A small 4 inch non-validated bar sealer is available. A larger validated sealer is available from Pack-World.

The temperature to seal FEP depends upon the pressure

III. CRYOPRESERVATION WITH KRYOSURE® BAGS

applied. With more than 40 pounds per square inch, the temperature may be as low as 280 °C. If the pressure is in the range of one pound per square inch, the temperature may be as high as 330 °C.

For non US markets:

Please contact CellGenix.

8. How can inflation be avoided?

First, avoid having any air bubbles in the KryoSure® bag at time of freezing. Second, avoid having excessive amounts of dissolved carbon dioxide in the solution at time of freezing. This is done by freezing as soon as possible to reduce cell metabolization and gas release.

9. Would it be better to set the freezer to –60 °C to avoid the gas thawing problem?

No, freezers that are made to run at –80 °C should not be set to run at any temperature warmer than –80 °C. At higher temperatures, the two refrigeration pumps in these „two-stage“ systems will be obliged to work against each other and one will fail.

10. Do you have special bags for freezing such as in nitrogen?

ULTRA COLD: All fluids can be safely frozen in liquid nitrogen in KryoSure® freezing bags. FEP remains flexible and stretchable at liquid nitrogen temperatures. FEPs glass transition temperature is –208 °C. FEP remains flexible at –196 °C. FEP is not only flexible, but elastic; it can stretch while in liquid nitrogen. The inability to stretch is the principle reason that other plastics break in liquid nitrogen service.

THERMAL TRANSFER: KryoSure® FEP bags are 0.127 millimetres (0.005 inches) thin for good thermal equilibrium during freezing. FEP is transparent for easy visual inspection during thawing.

FEAR OF BREAKING: Over the years, lesser bags have earned the reputation that a percentage of them will break in the liquid nitrogen, contaminating the liquid nitrogen with pathogens from the frozen cells.

BREAKAGE ON THAWING: Actually, lesser bags will more frequently break on thawing; causing loss or contamination of the valuable frozen cells.

11. Could KryoSure® bags break?

SLICING: Sharp ice edges cut the bag while freezing or while being handled. This is virtually the only mechanism of concern in FEP bags.

MECHANICAL: Ice expands; stretching the bag or its fittings beyond their elastic limit while freezing. This is not an issue with FEP because FEP remains elastic even in liquid nitrogen.

PNEUMATIC: Gas expands in the bag while thawing, and a non flexible bag breaks rather than stretch or bend. FEP just stretches.

12. Why don't KryoSure® bags break when they are removed from the freezer?

KryoSure® Bags stretch as they are removed from the freezer as the once frozen gas begins to fill the bag. The gas is carbon dioxide. The carbon dioxide was dissolved in the cryopreservation media as a result of the normal respiration of the cells metabolising sugar and producing carbon dioxide. The carbon dioxide is liberated from the aqueous solution as a gas bubble when the bag is removed from the freezer. Upon thawing, the gas, now occupying more space, expands within the bag. The amount of expansion relates to the amount of carbon dioxide dissolved in the solution at time of freezing. The volume of the gas will expand with temperature as the frozen sample thaws and at about –70 °C, the frozen CO₂ will thaw and return to a gas and expand. If the bag is a KryoSure® FEP container it will expand and (not perceptively) inflate. If the bag is made of other materials, the container will fracture because it is not flexible at that temperature. At cold temperatures the FEP becomes impermeable to gas transfer.

13. Will KryoSure® FEP bags break in immersion liquid nitrogen storage?

No, the reason that most plastic bags break in liquid nitrogen is because they become brittle in the cold, and rather than bend, they break. FEP remains flexible in liquid nitrogen. Even if bent, bashed, or flexed, FEP bends and springs back the same as at room temperature, accordingly it will not break.

14. Is it necessary to get all the air out of the fluid stored in the KryoSure® bag when placing it into liquid nitrogen?

It is desirable but not necessary. Any air in the fluid will make a bubble when the rest of the fluid freezes. As the temperature drops to $-182\text{ }^{\circ}\text{C}$ even the oxygen in the air will condense to a liquid leaving a vacuum in the bubble. When warmed, the oxygen will expand and fracture bags of less quality. The KryoSure® FEP bag will expand and stretch because it is still flexible and stretchable. The swollen bag may cause the cassette to be hard to open but the bag will not break.

15. What types of over wraps (KryoSure® FEP pouch over wrap versus Kapton/FEP peel pouch KP-0512) should be used to protect KryoSure® bags?

Over wraps may be used to protect KryoSure® bags from potential contaminants coming from the outside such as the nitrogen tank. Both can be used, KryoSure® FEP pouch over wrap and Kapton/FEP peel pouch KP-0512. It depends on the purposes of the customer. Orange over wraps can be peeled apart with hand strength. Clear FEP over wrap pouches must be cut open with scissors. Both types of over wraps are sealed closed with a high temperature bar sealer. If the customer does not have to peel the bag with hands and drop the contents onto a sterile field, one can use KryoSure® FEP pouch over wraps which are less expensive than the Kapton/FEP peel pouch KP-0512. The over wraps have to be ordered separately.

16. Can liquid nitrogen go through the FEP film of KryoSure® bags once the bag is immersed into liquid nitrogen?

KryoSure® bags made of FEP are very permeable to nitrogen, oxygen, and carbon dioxide at room temperature but at lower temperature the permeability is less. You will see that when immersed in liquid nitrogen there is zero permeability to nitrogen. That is because the permeability to nitrogen as a liquid is zero due to the difference between a liquid and a gas. Also at this low temperature the FEP material is less permeable to anything. Permeability is a function of temperature. (Also note that oxygen becomes a liquid at $-181\text{ }^{\circ}\text{C}$ and carbon dioxide becomes a solid at $-76\text{ }^{\circ}\text{C}$ so when these are no longer gasses, they are not permeable below those temperatures either). The conclusion is that in liquid nitrogen, nothing is permeable to the bags because permeability applies to gas not to liquid. So KryoSure® FEP bags are just as good at preventing intrusion of nitrogen as Kapton bags in liquid nitrogen. There is no "migration" of nitrogen through either material at liquid nitrogen temperatures.

IV. USE OF VUELIFE® ADHERENT CELL BAGS

1. What is a VueLife® AC bag and what is it for?

The „AC“ stands for Adherent Cell. The inside surface of the VueLife® AC bags have been modified by corona discharge treatment to permit adherence of cells or any non aqueous material which can be introduced into the bag (see instruction sheet in the part „How to use VueLife® AC bags“ of this document). VueLife® AC bags may be used to culture cells that require an adherent surface such as dendritic cells, and can also be used to immobilize cells.

2. Are the adherent properties strong enough to collect monocytes from buffy coats?

CellGenix is not able to test laboratory parameters for cellular therapy biologics and therefore don't know all the capabilities. We would appreciate any customer feedback for adherent experience with cells or other proteins.

In addition, caution must be used to keep the inside of the VueLife® AC bag touching the opposite side at all times until filling. Adherence will be negated by exposing the inside surface to air for any length of time. The VueLife® AC bags can be used for cell culture, U.V irradiation, and I.R. irradiation.

NOTE: The adherent surface must be handled carefully; otherwise the adherent properties are lost. See the instruction sheet below that is included with each order.

3. Is it possible to separate monocytes from mononuclear cells by using VueLife® AC bags in combination with DC Medium in advance?

Adherent properties of VueLife® AC bags are not strong enough to collect monocytes from buffy coats but it should work for mononuclear cells collected by leukapheresis. Products of leukapheresis are cleaner; they contain less contaminating components that can interfere with mononuclear cell adherence.

4. Instructions for use of VueLife® AC bags having an activated surface

- The surface of the inside of these VueLife® AC bags has been modified to permit adherence.
- The bags are supplied with the inside surfaces adhered together. If the bag is not stuck together, do not use it.
- Do not permit air to contact the inside surface of the bag.
- Do not flush the bag before use. There are no foreign materials in the bag.
- Do not add anything to the bag before adding the materials that are to be adhered to the surface.
- The first non-aqueous material in the bag will adhere.
- Fill with the bag hanging down so that any air stays at the top and does not contact the surface of the bag. If air contacts the interior the ability to adhere will be lost.
- It may be necessary to squeeze the materials into the bag. Use of the sterile docking transfer set would be helpful.
- Add the desired materials to the bag at temperatures above 25 °C. Stronger adherence is obtained at higher temperatures. Adherence is complete within 10 minutes.
- Do not permit the inside of the bag to dry after coating.
- If an intermediate protein is used such as retronectin or fibronectin, add the next phase as soon as possible after adhering the nectin.
- Materials can be removed from the surfaces of the bag by permitting sterile air to contact the surface (draw air into a syringe through a 0.2 micron filter). As soon as the surface is dry, adherence is reversed. The formerly adhered materials will be in solution, not on the surfaces.

5. How much retronectin should be used to coat the inside VueLife® AC bags?

A minimum of 5 µg / cm² of bag surface should be used. Keep in mind that enough retronectin must be added to coat both top and bottom of the interior of the bag. Accordingly add enough retronectin for both surfaces. It is believed that best transduction results are obtained if the retronectin is allowed to attach for two hours at room temperature or 30 minutes at 37 °C.

6. Is it possible to make silicone rubber bags?

While silicone rubber has good oxygen permeability if made thin enough and if made from the proper materials, it is also permeable to water. The constant loss of water from culture bags increases the osmotic pressure of the media and inhibits cell growth. Silicone rubber is not as permeable to carbon dioxide, accordingly acidity builds up and you cannot see through silicone rubber bags to detect the colour change.

7. Removing adherent cells from VueLife® AC bags

Cells should release from the surface of the bag once they are in contact with the air. You have to fill the VueLife® AC bag with some air using a syringe (a 0.2 micron filter may be attached to the syringe to draw the air through) and by slightly moving the bag, let the bubble of air go all the surface of the container. Then, by moving the container again, let the medium left in the bag go all over the surface of the VueLife® AC container so that the cells which do not stick to the surface of the bag anymore, but rather get resuspended into the medium. You could also remove the cells by gentle agitation of the container once the air bubble is inside. Then you have to transfer the medium that contains the cells to another container, centrifuge it and resuspend the pellet of cells in the medium of your choice. It may also possible to just completely take the medium out of the bag (to save it just in case some cells would be in it) and refill the bag with the medium in which you want to retrieve your cells. The cells may become unstuck and would get resuspended into the medium. Please note, this is not an exact science and user experimentation should proceed with purposeful caution.

8. What types of cells have adherence in VueLife® AC bags?

It should work for MSC. CellGenix believes that it may work as well for the adherence of the monocytes as long as they do not come from a buffy coat which brings to many contaminating components which can prevent cells to adhere, but leukapheresis products which are cleaner (contain fewer components of other cells).

9. How many cells can grow in a VueLife® AC bag?

It depends on the type of cell and type of feeding. Cord stem cells can expand many fold, some report 150 fold expansion. Unfortunately we don't have hard numbers of start and finish at this time. With T cells, people start with 1×10^6 cells per ml or more, depending on. A few fold or more expansions, depending on variables in a static bag like VueLife® / VueLife® AC, have been reported and could be realized.

V. VALIDATION OF VUELIFE® / VUELIFE® AC BAGS

1. Have your bags ever been approved for anything?

AFC bioprocessing containers have been FDA 510(k) cleared for culture or storage of leukocytes, for storage of tissue. This does not constitute approval for sale to contain other biologics or pharmaceuticals. A list with all standard and CE-certificated bags will be released soon. AFC also has device master files with the FDA and cross-reference permission may be requested in writing.

2. Are your bags approved for working with a human cell line that maybe used in immuno - therapy?

If your biologic is approved, the bag may be eligible for approval, but your biologic must be approved first (or simultaneously). The bag can only be approved with a given biologic if the biologic is approved and if the bag does not adversely affect the biologic.

3. Is there a validation procedure for KryoSure® bags?

Yes there is, for further information please look at www.toafc.com.

4. Why do bags need regulatory approval?

The bag may affect the safety or effectiveness of the biologic that is contained inside the bag. Accordingly, the bag can be approved for containing the biologic if it is proven that the container does not affect the biologic. For example, plasticizers in some containers may leech into the biologic and affect the biologic by changing the nature of the biologic. One example is DEHP which is used to plasticize polyvinylchloride bags. FEP bags do not contain any plasticizer. And for another example, some bags may react with the biologic or bind the biologic and thereby reduce the amount of the biologic in solution. An example might be of bags containing styrene which readily binds cells and cytokines. Bags made of FEP do not react with or bind anything.

5. Can I use VueLife® / VueLife® AC / KryoSure® FEP bags in my research?

In research, yes you can. But not if you are treating or diagnosing disease, unless you obtain approval by a Human Investigational Review Board, and you will probably need an Investigational New Drug Approval, and Investigational Drug or Device Exception, and you may need other approvals.

VI. GENERAL INFORMATION ABOUT VUELIFE® / VUELIFE® AC / KRYOSURE® BAGS

1. What is the difference between FEP and Polyolefin bags?

Polyolefin is the umbrella term for Polyethylene (= Polyolefin from Ethylene) and Polypropylene. You could find the characteristics of these substances here: http://www.corning.com/Lifesciences/technical_information/techDocs/propertyplast.asp

- the temperature range is smaller than for FEP
- PEP is not autoclavable, Polypropylene only up to 135 °C
- Polyolefin-bags do not have the tensile strength of FEP Bags, and they may break break at 2kp. Polyolefin bags could contain plasticizers.

2. What is the advantage of using FEP instead of EVA (ethyl vinyl acetate)?

CellGenix offers FEP bags for cryopreservation because FEP remains flexible in liquid nitrogen and is therefore much more reliable than any other plastic material. All other plastics become brittle even at –42 °C. When at temperatures below –80 °C, bumping the cassette can break an EVA bag frozen inside. By contrast, an FEP bag frozen at –196 °C in liquid nitrogen can be dropped on the floor without danger of breaking the bag. When the contents of the bag are very important, the risk of bag breakage is virtually eliminated by using FEP bags. In addition, FEP is transparent, and FEP has no extractables and no leachables.

3. Which bags are best for transduction / gene transfer of cells?

1. Transduction is best with VueLife® AC bags. You cannot coat retronectin on standard bags.
2. Most researchers are starting their cultures in 2PF-0072-AC bags.

4. How could VueLife® bags be centrifuged?

All VueLife® bags can be centrifuged. But you must follow certain rules:

First: The bag must be supported on the sides so that centrifugal force will not cause the sides to stretch. If the fluid in the bag will cause the bag to become stretched out at the sides, then the sides must be supported to avoid the stretching. This will happen if the centri-

fuge holder is too big for the bag. The bag should fit in the holder with the sides of the bag touching the sides of the holder all the way around. Some bags require a second bag in the same holder.

Second: The tubing or fittings must be folded against the side of the holder and be placed between the holder and the bag. If the fittings remain on top of the bag during centrifugation, they will puncture the bag and settle to the bottom of the bag.

Third: Fittings must not be under the bag. If fittings are under the bag, the bag will press around them and may cause a hole.

Fourth: The fittings must be tight. Pressure develops in the bag and the fittings will leak if not very tight.

Fifth: Place the bag in the centrifuge to avoid wrinkles in the bag. Wrinkles will trap the cells, preventing some cells from reaching the bottom pellet. Wrinkles become hard to remove after centrifuging. Try to have the bag match the size of the centrifuge holder and not be too large because if the bag is too large (or the holder too small) wrinkles will form.

Sixth: Wrinkles will form anyway. Because the bags are not the exact shape of the holder, the bag must have wrinkles someplace. Select a holder that gives the least wrinkles. Some of the holders that are made for „centrifuge tubes“ are very good for this; particularly the holders that are for 150 ml and 250 ml, „centrifuge tubes“ are good. CellGenix has a special centrifuge holder (VueLife® bags CI-50 for Centrifuge Insert- 50 ml) and bag that fits in centrifuges in the place where 96-well plates fit. This holder has a cover and can be used in P-3 (pathogen laboratories) applications. It holds a special bag (1P-0045) that fits in that holder to keep the bag flat during centrifugation. It is used for spin inoculation gene transfer. The bag can contain from 10 – 50 ml of fluid. It can be centrifuged at 4000 x g for hours at a time.

Seventh: Bags are OK for centrifugal forces below 1500 x gravity. If using higher G force, be very careful to support the bags.

5. DMSO & VueLife® bags

VueLife® bags are inert to DMSO. VueLife® fittings are also inert. That means that DMSO will not dissolve the bag or change it in any way. You can store DMSO in VueLife® bags for months or years and there will be no problem with the bag or the fittings. The tubing is a problem. The tubing will dissolve in DMSO. You can transfer DMSO in the tubing if the exposure is only for a few minutes, like less than 15 minutes.

VI. GENERAL INFORMATION ABOUT VUELIFE® / VUELIFE® AC / KRYOSURE® BAGS

It also helps if the DMSO is cold. Most customers always keep the DMSO cold when transferring it anyway. All tubing that works in the sterile docking device will also dissolve in DMSO. For many years, since 1965 people have used the tubing without problem because they keep the tubing and DMSO cold (about 4 °C) and they work quickly. If the DMSO is less than 25 % it dissolves plastic very slowly. Most people work with less than 20 % DMSO, so it is usually not a problem. But the Vuelife® bags are ok at any concentration.

PRODUCT LIST

Bioprocessing Containers – GMP

Product & Description	Code	Volume
VueLife® Cell Culture Bags		
VueLife® 1PF-0002	3002	2ml / 1 bag
VueLife® 2PF-0002	3007	2ml / 1 bag
VueLife® 32-C	3300	32 ml / 1 bag
VueLife® 72-C	3301	72 ml / 1 bag
VueLife® 2-PF-0118	3040	118 ml / 1 bag
VueLife® 197-C	3303	197 ml / 1 bag
VueLife® 290-C	3304	290 ml / 1 bag
VueLife® 750-C1	3305	750 ml / 1 bag
VueLife® AC Cell Culture Bags		
VueLife® 32-AC	3330	32 ml / 1 bag
VueLife® 72-AC	3331	72 ml / 1 bag
VueLife® 2-PF-0118-AC	3140	118 ml / 1 bag
VueLife® 197-AC	3333	197 ml / 1 bag
VueLife® 290-AC	3334	290 ml / 1 bag
VueLife® 750-AC	3335	750 ml / 1 bag
KryoSure® Freezing Bags		
KryoSure® 6-F	3360	6 ml / 1 bag
KryoSure® 20-F	3361	20 ml / 1 bag
KryoSure® 60-F	3362	60 ml / 1 bag
KryoSure® 120-F	3363	120 ml / 1 bag
KryoSure® 180-F	3364	180 ml / 1 bag

The list of bags is a selection of available products. Divers VueLife® Tubing Sets and Overwraps as well as several accessories such as clips, adaptors, culture cassette/rack, etc. complete the product range.

Customized sizes on request. For more detailed information, please have a look at www.cellgenix.com

If there is any question left or you need further assistance, please do not hesitate to contact our Customer Service by phone: **+49 761 888 89-330** or by email: info@cellgenix.com



CellGenix GmbH
Am Flughafen 16
D-79108 Freiburg
Germany

Phone: +49 761 88 88 9 - 0
Fax: +49 761 88 88 9 - 800
Email: info@cellgenix.com
www.cellgenix.com

Bioprocessing Containers
are manufactured by:



American Fluoroseal Inc.
431-A East Diamond Ave.
Gaithersburg, MD. 20877
USA

Phone: +1 301 990-1407
Fax: +1 301 990-1472
Email: info@americanfluoroseal.com
www.toafc.com