HemaPrime™ Cryopreserved Leukopak
GMPrime™ Cryopreserved Leukopak

Product Description

A leukopak is a mononuclear cell-enriched leukapheresis product collected from normal peripheral blood. It is composed of a variety of blood cells including monocytes, lymphocytes, platelets, plasma, and red blood cells.

HemaCare offers two types of cryopreserved leukopak:

1. HemaPrime: designated for research use only (RUO)
2. GMPrime: meets the strict quality standards as defined by the FDA (21 CFR 1271) and AABB

The GMP-compliant cryopreserved leukopak (GMPrime) is processed within HemaCare's cleanroom facility under direct quality oversight and is accompanied with robust quality-assured documentation. Designed with scalability in mind, the GMPrime cryopreserved leukopak is collected and processed following the same consistent procedures as the HemaPrime cryopreserved leukopak, thus, allowing researchers to seamlessly transition from development to clinical trials and, ultimately, commercialization.

Sample Collection and Processing

Leukopaks are collected at HemaCare's FDA-registered collection center from healthy human donors who have consented under an IRB-approved protocol. For HemaPrime products, donors are pre-screened for a full infectious disease panel including HBV, HCV, HIV, HTLV, WNV, Trypanosoma cruzi, and Syphilis 30 days prior to the collection as well as on the day of collection. GMPrime donors are pre-screened for the same full infectious disease panel in addition to CMV and Zika.

The leukopak is extracted by leukapheresis using the Spectra Optia® Apheresis System CMNC collection protocol directly into a bag containing ACD-A anticoagulant. The leukopak is quickly cryopreserved via a closed-system process on site in CryoStor® CS10 (GMP-grade freeze media, BioLife Solution, Seattle, WA) using an internally developed process for controlled-rate freezing.

Prior to freezing, approximately 65-70 mL of the leukopak with freezing media is transferred to a CryoStore™ 250 EVA Freezing Bag (CS250, OriGen Biomedical, 30-70 mL, standard tube set with two female luers with caps). The tail from the transfer pack is heat-sealed and not long enough for downstream sterile welding. The total pre-cryopreservation cell yield per transfer pack ranges from 2.0-2.5 x10^9 white blood cells.

Storage and Handling

Upon receipt, the cryopreserved leukopak can either be kept in a liquid nitrogen container for long-term storage, or it can be thawed for immediate downstream processing. The frozen bag is very fragile when removed from the liquid nitrogen container and we recommend handling it with care to avoid breakage.

Thawing

A complete thawing protocol of the cryo leukopak can be found on our website (www.hemacare.com/resources/).

When thawing the cryopreserved leukopak, we recommend placing it into a 37°C water bath. Water should be in contact with as much of the bag as possible without compromising the potential sterility of the desired transfer method (tubing or dedicated port). If the water level is not high enough, it is permissible to tilt the bag to ensure that the majority of the bag is in contact with the water. Ideally, the water level should be just below the transfer ports which are located at the top of the bag and are facing upwards. When thawing the leukopak, it is important to minimize the amount of movement, as the application of force to semi-frozen components can damage the cellular material. If repositioning or reorienting of the leukopak is required, it should be done slowly.

Thawing media can be added directly to the leukopak bag through one of the available ports found on the bag using a plasma transfer set or anything that connects to the luer lock. Once the bag is thawed, it can fit up to 250 mLs of liquid before becoming turgid. More thawing media is okay, but we have found good results with a simple 1:1 volume ratio.

Transferring

The thawed leukopak material can be transferred out of the bag using one of two routes:

1. **Through the heat-sealed section of the tubing.** Once the leukopak is thawed, this section of the tubing can be cut off and contents of the leukopak can be gently drained into a container. Even though this procedure makes it difficult to wash the inside of the bag in order to maximize recovery of cells, it requires no extra parts and it is the least involved way to empty the leukopak bag.

2. **Through the two identical ports.** These are standard laboratory size to accept an adapter. Easy options include a plasma transfer set or equivalent. The ports are bisected into a top and bottom portion. The top of the port can be twisted to make it come off. This exposes a path by which to attach a transfer set. The transfer set still has to pierce an internal plastic membrane within this port. Make sure when attaching a transfer set that it pierces the membrane completely to allow for proper fluid flow.

Washing

After the contents of the leukopak have been drained, it is advisable to wash the bag with more thawing media in order to maximize cell recovery after cryopreservation. The volume of thawing media used for this wash step should be >10 mL for good wash efficiency.

Centrifugation

After thawing media is added and contents of the leukopak are poured/aspirated out of the ports, centrifugation will be necessary to remove cells from the aqueous DMSO. Transferring the leukopak material to a conical tube is preferred for the centrifugation step.

In general, we recommend centrifuging the cell suspension at 350-400 x g for 10 minutes at room temperature. However, the centrifugation speed and duration can vary depending on the cell and conical tube types. When optimizing the centrifugation parameters for downstream applications, we advise finding a balance between the following two factors:

1. Pelleting the majority of the cells without damaging them at high centrifugation speeds. This directly influences the ability to recover cells from the leukopak.

2. Finding the appropriate centrifugation speed with the least amount of impact on the cells, which typically indicates using lower speeds. Using a lower centrifugation speed will also prevent pelleting down debris, which is significantly less dense than cells. This can often solve problems relating to dead cells, platelets, or other debris.

It should not be of concern if the supernatant is tinted red after centrifugation. This color is due to red blood cell hemolysis, which is an expected result of cryopreservation and thawing.

Straining

After centrifugation, the cells can be resuspended in any media desired for the downstream work. If the downstream work is possibly affected by a non-heterogenous mixture of material, it is important to filter out large debris or clumps. Clumping is a natural result of the unavoidable but minimal cell death that occurs during our cryopreservation process. The exogenous DNA from the lysed cells is sticky and can lead to clumps which may impact certain types of assays or downstream procedures. This is especially important when dealing with any sort of column-based separation platform, as clumps will impact the flow rate of the material through the column. Another assay where straining the material is beneficial would be for antibody-based labeling incubation. The clumps can often interfere with the interaction of the antibodies with their intended targets, leading to either incomplete or non-specific binding.

Cell strainers are an excellent choice to remove any unwanted clumps and choosing the right pore size is important. We find that for immunological-type assays, 40 μm cell strainers are generally the standard. However, choosing the optimal strainer type will ultimately depend on the end user's application.

Shipping Condition

The cryopreserved leukopak is placed inside a protective metal cassette and shipped in a returnable liquid nitrogen dewar.

Downstream Applications

- Isolation of mononuclear cells (PBMCs) via density gradient.
- Isolation of specific mononuclear cell subtypes (example: T cells, B cells, NK cells, monocytes, etc..) using immunomagnetic separation.
- General characterization of cellular subtypes via FACS.

Note: For functional downstream work, there is evidence throughout the relevant literature that resting the cells overnight under certain conditions is beneficial and/or necessary for the proper function of the cells. However, such assays will require further optimization on the user end as they are protocol dependent.

Leak and Spill Procedures

1. Wear chemical-resistant groves.
2. Absorb spill and place in a closed container for disposal.
3. Wash area thoroughly after clean-up is complete.

Hazard Identification

The leukopak is a human-derived biological material. Although the donor has been screened against and found to be negative for HBV, HCV, HIV, HTLV, WNV, Trypanosoma cruzi, and Syphilis (and Zika for GMPrime), all materials which have come in contact with this product must be treated as infectious materials. Therefore, regulations for the treatment of infectious materials must be observed when handling this product.

Universal or biosafety level 2 laboratory precautions must be utilized when working with the leukopak, and trained personnel observing good laboratory practice should handle the product. Always wear protective gloves, eye protection, and a lab coat when handling this product. Avoid skin contact or swallowing.

Emergency Overview	Mild irritation might result with eye contact and/or ingestion. Although the donor has been screened against and found to be negative for HBV, HCV, HIV, HTLV, WNV, Trypanosoma cruzi, and Syphilis (and Zika for GMPrime), the sample should still be treated as potentially infectious. Usage of gloves, eye protection, and laboratory coat is highly recommended when handling this product.
OSHA Hazards	This substance contains no ingredients at concentrations to be considered hazardous as defined by OSHA 29CFR 1910.1200.
Target Organs	Eyes, skin
Primary Route(s) of Entry	Skin, eye contact, inhalation, and ingestion
Symptoms of Exposure	Mild irritation.
Inhalation	May cause irritation to mucous membranes and upper respiratory tract.
Eye Contact	May cause mild irritation.
Skin Contact	May cause mild irritation.
Ingestion	May cause irritation to gastrointestinal tract and adverse health effects.

First Aid Measures

Report to your Safety Office and seek medical attention as soon as possible.

Skin Contact	Wash thoroughly with copious amounts of water. If skin is broken, seek medical advice.
Eye Contact	Hold eye open. Flush with copious amounts of water for at least 15 minutes.
Inhalation	If person is unconscious, seek emergency medical attention. If person is conscious, move to fresh air and call a physician if respiratory complications arise.
Ingestion	If person is unconscious, seek emergency medical attention; never give anything by mouth to an unconscious person. If the person is conscious, wash mouth out with copious amounts of water and call a physician. Do not induce vomiting unless directed to do so by a physician.

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