

Protocol for Expansion of T Cells in VueLife® “HP” Series Bags from 50-HP to 200-HP to 1000-HP

VueLife® “HP” Series Bags are Saint-Gobain Life Sciences’ “high-permeability” FEP bags for *in vitro* bioprocessing applications. Applications include the culture of highly concentrated immune cells, such as T or NK cells that require higher gas exchange rates for efficient culture and/or expansion *in vitro*.

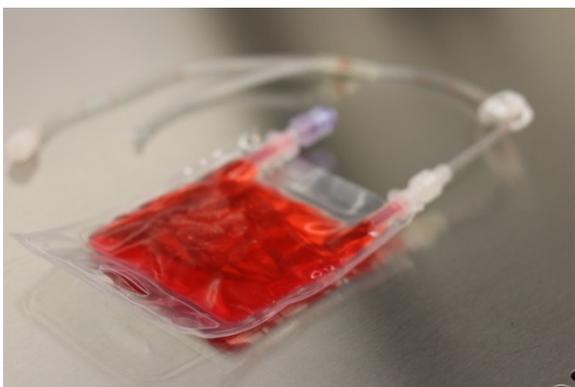


Figure 1. VueLife® “HP”-Series Bag

The novel “HP” laminate provides elevated oxygen and carbon dioxide permeability to the container, introducing further enhanced gas exchange capability to the VueLife® product line. The fluid contact-layer remains the same material as used in the VueLife® “C” Series range, harnessing the superior material properties of FEP. Each “HP” Series bag typically features a needleless injection site and “Y” connector with 7” PVC tubing leading to a female luer and a heat sealed sterile docking tube.

Expansion of T cells in VueLife® “HP” Series Bags: from 50-HP to 1000-HP

This document provides a step-by-step protocol and description for the expansion of human T cells in VueLife® “HP” Series Bags. This document is intended to be an illustration for the user and

meant to provide an example for use, and reflects our experience and practices gained in our internal lab.

Many variables can affect T cell expansion, including starting cell populations (T cell subsets), cytokines, and growth factors. Thus, users will need to investigate the culture conditions that will provide the optimal result with their specific protocols.

Protocol for T Cell Expansion in VueLife® HP Series Bags using OpTmizer™ medium and Dynabeads

List of Materials

Product	Manufacturer	Catalog #
50-HP	Saint-Gobain	50-HP
200-HP bag	Saint-Gobain	200-HP
1000-HP bag	Saint-Gobain	1000-HP
OpTmizer™ bottle	Gibco	A10221-01
OpTmizer™ media bag	Gibco	A10221-03
OpTmizer™ supplement	Gibco	A10484-02
L-Glutamine	Gibco	25030-081
Human IL-2 IS	Miltenyi Biotec	130-097-748
Dynabeads™ CD3/CD28	Gibco	11132D
Leukopak	Miltenyi Biotec	150-000-452
StraightFrom® Leukopak® CD4/CD8 MicroBead Kit	Miltenyi Biotec	130-122-352
Running buffer	Miltenyi Biotec	130-091-221
PBS	Gibco	14190-144
Terumo TSCD-II Welder	Terumo	TSCD-II
Tube sealer: Rapid Seal II	Genesis	Se340
Spike Port Connector	Saint-Gobain	MLSP-14-RC

Separation of T cells

Separate CD4⁺/CD8⁺ T cells from Leukopak using the StraightFrom[®] Leukopak[®] CD4/CD8 MicroBead Kit (Miltenyi Biotec), following manufacturer's recommendations.

Seeding of T cells in 50-HP bags and Activation via Dynabeads™

For the initial seeding step, we seeded 50x10⁶ cells in a total of 50 mL volume (=1x10⁶ cell/mL). We added Dynabeads™ at a bead: cell ratio of 1:1. These are the detailed steps:

- 1) Prepare Dynabeads™:
 - Vortex Dynabeads for 30 seconds and transfer 1250 µL into a conical tube. Add 2 mL of wash buffer and vortex for 5 seconds.
 - Place the tube into the DynaMag™ for 1 min and remove the supernatant via pipette.
- 2) Mix Dynabeads™ and T cell: Transfer 50x10⁶ T cells into a sterile conical tube together with Dynabeads™ and mix well by pipetting.

Transfer the mixture into the 50-HP bag prefilled with 40 mL of culture media and wash the tube with the appropriate volume (~10 mL) of media to bring total cell culture volume to 50 mL.

Expansion of T cell cultures & Transfer cultures to larger bags

When viable cell density exceeds 1x10⁶ cells/mL, the cell culture should be expanded by addition of fresh media and transfer to a larger bag, if needed. We recommend to fill the bags to 1 cm height, as this has shown to support optimal cell performance in our studies.

We recommend dilution of the cell culture to 0.5x10⁶ cells/mL but no lower than 0.3x10⁶ cells/mL. In our experience, this is the lower limit to ensure cell survival and consistent proliferation.

- 1) Day 3 of culture: Depending on the cell concentration, T cells can be maintained in the 50-HP bag or transferred to a 200-

HP bag. We recommend to use at least 100 mL of culture volume in the 200-HP bag.

- 2) Day 5 of culture: For further culture, T cells can be maintained in the same 200-HP bag, and culture volume can be increased to 200 mL, or cell cultures can be split and transferred into other, multiple bags if desired.
- 3) Day 7 of culture: Transfer culture from 200-HP bag to 1000-HP bag. Fill the 1000-HP bag with 800 mL of fresh medium by weight, and add the 200 mL of culture volume.
- 4) Day 10 of culture: Harvest cells and perform final cell characterization, quality control and process as intended.

Sampling the culture

- 1) Remove the bag from the incubator and gently invert the bag several times to homogenize the culture for accurate sampling.
- 2) Collect a sample by attaching a syringe to the needless valve port and removing the appropriate amount.

Note: We recommend pipetting the sample up and down a few times to disperse any cell aggregates. You may also want to de-bead the sample on the DynaMag™ to avoid counting the beads in your sample.

Adding culture media from bags to VueLife[®] bags

To connect Thermo Fisher media storage bags to VueLife[®] culture bags, we recommend using Saint-Gobain Life Sciences' spike port connector (MLSP-14-RC). This part can be used to connect the media bag via its spike port and Saint-Gobain Life Sciences' VueLife[®] bag via the Luer lok (Figure 2).



Figure 2. Addition of culture media from media bag to VueLife® culture bag. Bag contents can be transferred via gravity and volume can be tracked by using a lab scale.

Connecting bags using a sterile connection device

Cell cultures can be transferred from one bag to another via two methods: a) manual transfer via syringe and b) transfer via a sterile connection device.



Figure 3. VueLife® bags can be connected using a sterile connection device and welding the ends of the Y-tubing.

- 1) Connect the Y-tubing on the bags by welding, as shown in Figure 3.
- 2) Cultures can be transferred by gravity and cultures volumes can be tracked using a lab scale.

- 3) After the transfer is complete, bag can be separated by sealing the tubing using a tube sealer and disconnecting the tubing.
- 4) To ensure complete transfer of all cells from the source bag to the recipient bag, we commend washing the source bag with culture medium, e.g. by addition of fresh medium to the 50-HP bag and then transfer via tubing to 200-HP bag.

Removal of air from VueLife® bags

Remove air from the culture bags by attaching a sterile syringe to the needless valve port and drawing up the air into the syringe. Alternatively, a vacuum pump and sterile filter (we use Sartorius™ S6596FMOSK) can be used.

Please contact us if you have any questions related to the information presented here. We are continuously working to improve the use of our products.

ABOUT

SAINT-GOBAIN

Saint-Gobain Life Sciences is proud to take part in providing solutions for a multitude of cell therapy applications while collaborating with customers and industry partners to develop custom disposables, often for integration into automated systems. Through our material science expertise as well as our deep experience in bringing manufacturing technologies to scale, we are uniquely positioned to offer solutions to the numerous challenges faced by cell therapy manufacturers today.