

# Coating VueLife® Bags with RetroNectin®

Transduction is the process by which foreign DNA is introduced into a cell by a viral vector and is commonly used in production of CAR-T cell therapies. Some cell types are particularly difficult to infect, even with highly concentrated lenti- or retroviral vectors, including hematopoietic cells such as primary T cells. Transduction mediators are often used to promote binding and entry of viral vectors into cells.

RetroNectin®, a recombinant fibronectin fragment from TakaraBio, is one such transduction mediator. It is widely used to coat cell culture surfaces to increase transduction rate. This recombinant protein has functional domains which aid in the co-localization of target cells and viral vectors. By facilitating close proximity, the RetroNectin reagent can enhance viral-mediated gene transfer to target cells.

VueLife® bags are closed cell culture systems used in the cell therapy industry for expanding T cells. In this technical bulletin, we demonstrate how to coat VueLife® 32-C and 32-AC bags with RetroNectin®. After coating VueLife bags with RetroNectin, the transduction step can then be performed in the coated bags. The details of performing transduction in VueLife bags can be found in the Technical Bulletin: “T Cell Transduction with Concentrated Lentivirus in VueLife® 32-C/32-AC Bags.”

## VUELIFE® “C” AND “AC” SERIES BAGS

VueLife® bags are manufactured using the highest quality USP Class VI fluorinated ethylene propylene (FEP) and are highly permeable to oxygen and carbon dioxide, while being impermeable to water. VueLife® “C” and “AC” bags typically feature a needle-less injection site and a “Y” connector with PVC tubing leading to a female luer and a heat-sealed sterile docking tube. This reduces the risk of culture contamination while allowing easy access for the introduction of new material. Other types of ports, tubing, and connections are available and can be customized to the needs of the user.

VueLife® “C” series bags are designed using FEP film for culturing and expansion of suspension cells including T cells. The VueLife® “AC” series uses a treated form of FEP film. This proprietary surface treatment results in the VueLife® AC Bag having a higher surface energy than the VueLife® “C” Bag. The modified surface of the VueLife® “AC” Bag is intended to promote better cell and protein adhesion to the bag surface, a feature that can be leveraged for transduction and transfection applications.

## PREPARING FOR RETRONECTIN® COATING

### Materials

In addition to VueLife® 32-C and 32-AC bags from Saint-Gobain Life Sciences, the following reagents were used in the process of coating FEP bags with RetroNectin® solution for transduction applications:

Name	Source	Catalog #
RetroNectin®	TakaraBio	T100A/B
BSA	Sigma	A7906-50G
DPBS	ThermoFisher	14190144
20ml Syringe	BD	302830

**Table 1:** Reagents for coating VueLife® 32-C and 32-AC bags with RetroNectin®.

### Preparing RetroNectin® and BSA Working Solutions

To prepare the RetroNectin® coating working solution, the following factors should be considered:

- Bag surface area to be coated
- Desired RetroNectin® concentration per cm<sup>2</sup> of bag surface area (single side)
- Solution volume needed to fully cover the bag surface
- Starting RetroNectin® solution concentration

The area of one side of the VueLife® 32-C and 32-AC bags was calculated as 43.5cm<sup>2</sup>. For further information on the dimensions of standard VueLife® bags, please see <https://www.celltherapy.saint-gobain.com/products/cell-culture-processing-bags>.

For these experiments, the starting RetroNectin® solution concentration was 1µg/µl. The coating volume necessary to fully cover the surface of VueLife® 32-C and 32-AC bags was determined to be 15ml per bag. This volume should be adjusted according to the bag dimensions to approximately half the recommended use volume for a given bag size.

In order to setup for bag coating with RetroNectin®, the appropriate volume of concentrated RetroNectin solution was diluted in DPBS to make 15ml of RetroNectin working solution. **Table 2** below gives an overview of key details for RetroNectin working solution preparation for VueLife® 32ml bags over a range of desired surface coating concentrations, from 20µg/cm<sup>2</sup> down to 1µg/cm<sup>2</sup>. All RetroNectin working solution parameters should be adjusted for bag size.

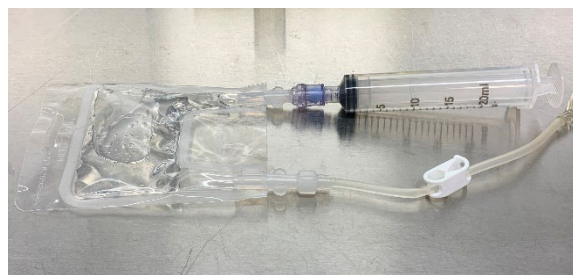
	Surface Coverage (µg/cm <sup>2</sup> )			
	20	10	5	1
Bag Area (cm <sup>2</sup> )	43.5	43.5	43.5	43.5
RetroNectin® / bag (µg)	870.0	435.0	217.5	43.5
Stock Solution Conc. (µg/µl)	1.0	1.0	1.0	1.0
Stock solution / bag (µl)	870.0	435.0	217.5	43.5
Coating Volume (ml)	15	15	15	15

**Table 2:** RetroNectin® working solution preparation for coating VueLife® 32-C and 32-AC Bags from high to low surface coverage.

The 2% bovine serum albumin (BSA) blocking solution was prepared by dissolving BSA in DPBS. The solution was then filtered with a 0.22µm syringe filter prior to use.

## RETRONECTIN® COATING OF VUELIFE® BAGS

Syringes were used to remove air from bags after each process step and to transfer fluid in and out through the needle-less injection site. Clips on the Y-tubes should be closed before adding any liquid to the bags (**Figure 1**).



**Figure 1:** Syringe attachment to VueLife® Bag to exclude air and transfer fluid in and out through the needle-less port.

After removing air from the bag, 15ml of RetroNectin® working solution was pushed into each VueLife® 32-C and 32-AC bag and incubated for 2 hours at room temperature or overnight at 4°C. The working solution concentration was dependent on the desired RetroNectin surface coverage on one side of each bag. Next, 15ml of BSA blocking solution is added to the bag after RetroNectin coating is completed and incubated for 30min at room temperature. The bags are then filled with 15ml DPBS and can be stored in the refrigerator for up to one week.

## BSA BLOCKING DISCUSSION

Our testing results indicate that there is no significant difference in transduction rate of human primary T cells with or without the BSA blocking step in both VueLife® 32-C and 32-AC bags. Please see the technical bulletin “T Cell Transduction with Concentrated Lentivirus in VueLife® 32-C and 32-AC Bags” for additional details.

## 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.