T Cell Transduction with Concentrated Lentivirus in VueLife® 32-C and 32-AC Bags

T cell engineering is a critical step in generating CAR-T cells for cell therapy applications. Genetic features of T cells are often modified via transduction using lenti- or retroviral vectors. Saint-Gobain Life Sciences' VueLife® bags provide a closed cell culture system to expand T cells for cell therapy applications. In this technical bulletin, we demonstrate successful transduction of T cells using a concentrated lentiviral vector in both VueLife® 32-C and 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 than can be leveraged for transduction and transfection applications.

MATERIALS & METHODS

Materials

In addition to VueLife® 32-C and 32-AC bags from Saint-Gobain, the following cells and reagents were used in the process of coating FEP bags with RetroNectin® solution followed by transduction of T cells with a concentrated lentiviral vector.

Name	Source	Catalog #
CD4+/CD8+ T cells	Miltenyi	150-000-452
DynaBeads™ CD3/CD28	Gibco	11132D
OpTmizer™ T Cell Expansion SFM Medium	Gibco	A1048501
L-Glutamine	Gibco	25030-081
IL-2	Miltenyi	130-097-748
RetroNectin®	TakaraBio	T100B
rLV.EF1.ZsGreen1-9	TakaraBio	0038VCT

Table 1: Reagents for performing transduction of T cells in VueLife® 32-C and 32-AC bags using concentrated lentiviral vector.

Preparing VueLife® Bags for Transduction

VueLife® 32-C and 32-AC bags were coated using RetroNectin® according to the protocol described in a separate technical bulletin, "Coating VueLife® Bags with RetroNectin®."

Isolation and Activation of T Cells

T cells were isolated from a Leukopak and frozen in 10% DMSO with culture media until ready to use. Thawed T cells were activated for two days in VueLife® "C" or VueLife® "AC" bags using anti-CD3 and anti-CD28 DynaBeads™ and expanded in OpTmizer™ culture media per manufacturer instructions. Media was supplemented with IL-2 at 200IU/ml. The cell-to-bead ratio was 1:1 with a T cell concentration of $1x10^6$ cells/ml at activation. Because the T cell population typically declines during the first two days of activation, an excess of T cells should be activated to have sufficient cells to perform transduction.

Additional information regarding activation of T cells in VueLife® bags is included in a separate technical bulletin, "Protocol for Expansion of T cells in VueLife® "HP" Series bags from 50-HP to 200-HP to 1000-HP."

Transduction in VueLife® Bags

After two days of activation in VueLife® "C" or "AC" bags, $7.5x10^6$ activated T cells were transferred to a 50-ml conical tube, centrifuged, and re-suspended in 15 ml of fresh media. In this protocol, DynaBeads™ were not separated from the T cells prior to transduction.

Lentivirus was supplied from TakaraBio as highly concentrated prepackaged lentiviral particles with green fluorescent protein genes for transducing mammalian cells. The lentivirus concentration ranged from 10^9 to 10^{10} TU/ml. Because the volume of lentivirus solution required was quite small, the viral vector was directly added to T cells in 15 ml of culture media for transduction. The volume of lentivirus solution added was determined based on the desired multiplicity of infection (MOI) as shown in Table 2:

	Target MOI			
	20	**10**	**5**	**1**
T Cells Number x 10^6	7.5	7.5	7.5	7.5
Virus Concentration x 10^9 (TU/ml)	7.0	7.0	7.0	7.0
Amount of Lentivirus Used (µl)	21.4	10.7	5.35	1.07

Table 2: Amount of lentivirus used for transduction based on target MOI and a viral vector solution concentration of 7.0 X 10^9 TU/ml.

The volume of lentivirus required depends on the starting solution concentration and number of cells to be transduced. The amount of viral vector used was calculated using the following formula:

$$\text{Viral Vector (µl)} = \frac{\text{Number of Cells Seeded}}{\text{Viral Vector Titer (TU/ml)}} \times \text{MOI} \times 1000$$

The T cell/lentivirus mixture was transferred via syringe into RetroNectin®-coated VueLife® 32-C or 32-AC bags, and all air was removed from the bags. The RetroNectin-coated side of the bag was placed downward on the incubator rack for the duration of the transduction process. T cells were incubated with lentiviral vector for two or three days before harvesting.

Harvest and Analysis of Transduced Cells

After two to three days of transduction, 1x10^6 cells were collected and washed with FACS buffer (0.5% BSA in DBPS + 2mM EDTA). Flow cytometry was used to quantify the percentage of transduced cells by GFP expression.

RESULTS

Transduction rate varies with viral MOI

Results of T cell transduction in RetroNectin®-coated (20 µg/cm^2) VueLife® 32-C and 32-AC bags demonstrated that higher transduction rates were achieved at higher MOIs after three days. The highest transduction rate, 86%, was achieved at the highest MOI, 20. Transduction rate then decreased with MOI and drastically declined at MOI 1. Similar results were obtained in VueLife® "C" and "AC" series bags. **Figure 1** provides an overview of transduction rates in both bag types across a range of MOI.

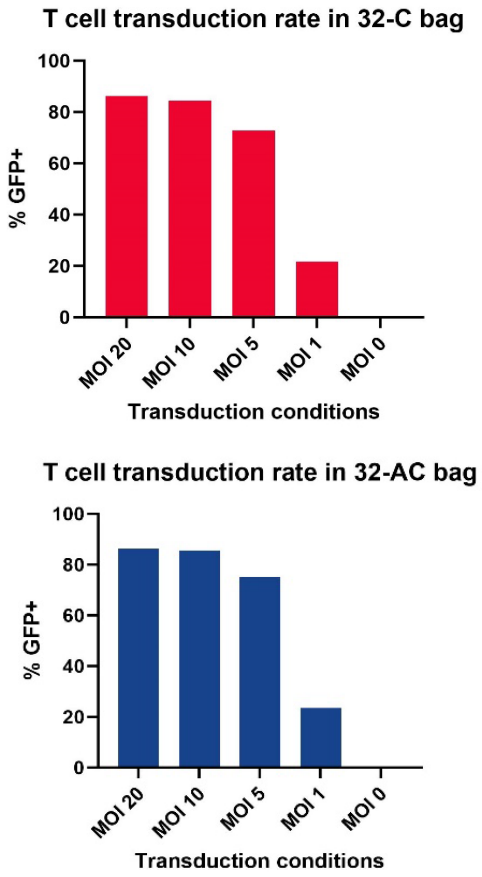

Figure 1: Higher T cell transduction rates were achieved with higher MOI in VueLife® 32-C and 32-AC bags.

RetroNectin® coating plays an important role in improving T cell transduction rate

Significantly higher T cell transduction rates were achieved in VueLife® 32-C and 32-AC bags coated with 20 µg/cm² RetroNectin®. In "AC" series bags, transduction rates of 76% were achieved with RetroNectin versus 49% without, and in "C" series bags, the transduction rate was 73% with RetroNectin versus 35% without. Of note, the transduction rate in "AC" bags not coated with RetroNectin was higher than in uncoated "C" bags.

The experiment captured in **Figure 2** below represents data collected following two days of transduction with lentivirus with an MOI of 10.

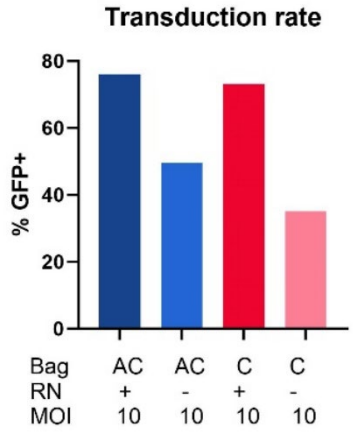

Figure 2: Higher T cell transduction rates were observed in VueLife® "C" and "AC" bags with RetroNectin® coating (RN; µg/cm²). Transduction rates were higher in "AC" bags than "C" bags in the absence of RetroNectin coating.

Furthermore, the effect of RetroNectin® coating concentration had a higher impact on the transduction rate when a lentivirus with a lower MOI was used to transduce T cells.

To understand the impact of RetroNectin® coating concentration, experiments were performed in VueLife® 32-AC bags with MOI of 10 and 5; RetroNectin coating was titrated from 1 µg/cm² (RN1) up to 20 µg/cm² (RN20).

As shown in **Figure 3**, there was no significant difference in transduction rate at MOI 10 with different RetroNectin® coating surface concentrations. The transduction rate was 65% at RN20 and 63% at RN1.

However, with MOI 5, transduction rate was found to decrease with RetroNectin® surface coating concentration from 57% at RN20 to 45% at RN1.

No significant difference in transduction rate with or without BSA blocking during RetroNectin® coating

A BSA blocking step is included as part of the standard RetroNectin® coating protocol from TakaraBio but was not found to significantly impact transduction rate in VueLife® "C" or "AC" series bags. Three experiments were performed in each bag type with 2% BSA blocking during RetroNectin coating and without the BSA blocking step. MOI 10 was used with two days of transduction.

The average transduction rate in VueLife® 32-AC bags with BSA blocking was 65% versus 63% without the BSA blocking step. In VueLife® 32-C bags, the transduction rate was found to be higher without BSA blocking at 66% versus 60% transduction with BSA blocking. **Figure 4** summarizes these results in VueLife 32-C and 32-AC bags with and without the BSA blocking step

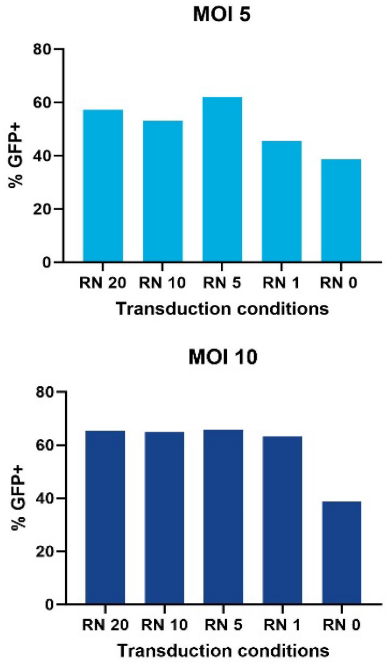

Figure 3: Transduction rate with different surface coating concentration of RetroNectin® (RN; µg/cm²) in VueLife® 32-AC bags at MOI 5 and MOI 10.

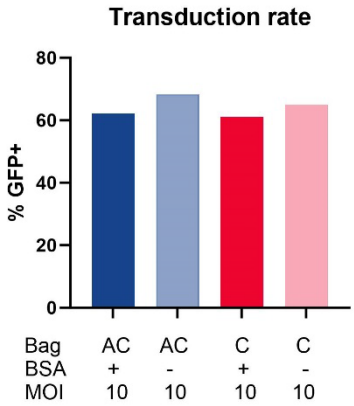

Figure 4: No significant difference in transduction rate with or without BSA blocking step during RetroNectin® coating in 32-C and 32-AC bags.

DISCUSSION & CONCLUSIONS

VueLife® "C" and "AC" series bags show excellent performance in T cell transduction facilitated by RetroNectin® coating. High transduction rates were achieved using high titer viral vector, exceeding 80% under some conditions. Because the lentivirus titer was high for each transduction experiment, very small volumes (µl) were required, so transduction was performed by simply adding the virus directly to the T cell culture.

If the viral vector used has a lower viral titer, a larger volume of solution would be required, to achieve the same target MOI. In this case, transduction cannot be performed by mixing the virus directly with the T cells because the volume may equal or even exceed the volume of the T cell culture. Further protocol development is currently underway with lower virus titer for transduction in VueLife® "C" and "AC" series bags.

The RetroNectin® coating plays an important role in improving the transduction rate of T cells. The impact of RetroNectin is dependent on the target MOI – at higher MOI, the impact of RetroNectin concentration is insignificant while at lower MOI there are obvious but not dramatic improvements with higher RetroNectin concentration. Generally, lower RetroNectin concentration can be used in VueLife® "C" and "AC" bags to reduce the cost of the transduction process. Furthermore, our results show that "AC" bags could be successfully used for transduction without coating, as the transduction rates in VueLife "AC" bags not coated with RetroNectin (49%) were higher than the transduction rates in uncoated VueLife "C" bags (35%).

Our testing results demonstrated no significant difference in transduction rate with or without the recommended BSA blocking step during RetroNectin® coating in VueLife "C" and "AC" bags. Based on this, the BSA blocking step may be skipped to save time and labor.

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.