New Technology Solutions for Lentiviral Vector Clarification

Appendix – Materials and Methods

A) Lentiviral vector production

Lentiviral vector is expressed using the CTS LV-MAX™ Lentiviral Production System (cat# A35684) at 1-10L stirred tank bioreactor scale according to the protocol outlined in the LV-MAX™ Lentiviral Production System User Guide.

Suspension format Viral Production Cells (VPC) are grown in a chemically defined culture system and are transiently transfected according to manufacturer's instructions using the proprietary transfection reagent, a production supplement, and an enhancer. Two days post-transfection, the bioreactor culture is subjected to harvest clarification using one of the two counterflow centrifugation systems followed by filtration through a 0.45 µm low protein binding filter or other equivalent method to further remove cell debris.

B) LV Clarification Using CTS Rotea System

CTS Rotea Kit Assembly and Setup

1. Culture medium from lentiviral production can be directly processed through a CTS Rotea system by harvesting directly into an appropriately sized transfer bag or filling a transfer bag with culture medium. To harvest directly into a transfer bag, sterile weld the transfer bag to the bioreactor and remove culture medium with a peristaltic pump.
2. Fill a separate appropriately sized transfer bag with pre-warmed LV-MAX production media (≤10mL/500M cells processed + 20 mL for priming). Volumes and appropriate bag sizes can be determined using the protocol simulator in the Rotea Protocol Builder software.
3. Attach lentiviral production culture medium, LV-MAX production medium, lentivirus reservoir, and cell output bags to a CTS Rotea single-use kit with a sterile welder.
4. Load assembled Rotea kit into Rotea instrument, open Rotea GUI software, and load LV Clarification protocol .json file. Determine appropriate data entry values using protocol simulator infectiousity of Rotea Protocol Builder software.

Table 2. Recommended data entry values for the Rotea software for LV clarification protocol

Data Entry	Recommended Values	Description
Empty Volume	18 mL	Priming volume as determined by protocol simulator that is run back out of LV reservoir bag to limit dilution of clarified lentivirus. May vary depending on the length of tubing.
LV Cell Draw Volume	100 mL	Draw volume of culture medium per loop – limited by chamber size, to be determined by volume of culture medium containing 500M viable cells.
Output Volume	10-20 mL	Volume of LV-MAX Production Media used to harvest collected viral production cells. Recommended minimum 10 mL volume used to fully clear chamber of cells and other debris. Larger volumes may improve turbidity reduction at the cost of increasing processing times.

CTS Rotea Protocol

This protocol is intended to elutriate LV while capturing LV MAX Production cells in the cone. The system is primed with LV MAX Production media prior to introduction of the virus and cell mixture. The priming volume is then run back into the output bag to minimize the dilution of the collected lentiviral vector.

Process Parameter Considerations

This protocol utilizes an additional empty step to limit the amount of dilution caused by excess priming volume. This volume can be determined by the user using the protocol simulator (Rotea Protocol Builder software) but will vary depending on the length of tubing used. Increasing this volume will decrease the amount of dilution but it is critical to avoid introducing air into the chamber since this can cause a significant instrument error.

Note that the Gibco VPCs can fill up the counterflow chamber quickly; therefore, users should aim to load no more than 5 x 108 viable cells at a time to effectively capture and separate cells from the LV supernatant. Additionally, the cells can settle quickly because of their size, which can result in inconsistent loading into the Rotea. Ensure that the input culture media bag is mixed well prior to and during the loading steps for consistent results.

C) LV Clarification Using Sartorius Ksep400 Counterflow Centrifugation System

Ksep400 Kit Assembly and Setup

The Ksep400 single-use consumable kits consist of a set of four radially installed conical centrifuge chambers (Ch) with a volume of 100 mL each and a tubing set.

1. Install the consumable kit into the Ksep400 hardware system.
2. Culture broth from lentiviral production can be directly processed through the Ksep400 system by connecting the bioreactor harvest port to the Ksep400 inlet tubing through sterile welding.
3. Attach washing buffer (i.e., PBS), a product reservoir bag, and a cell output bag (waste) to the Ksep400 tubing set with a sterile welder.
4. Load the appropriate LV Clarification protocol file. See Table 2 below for recommendations of process parameters.
5. Start clarification.

Table 2. Recommended starting parameters for LV vector clarification using the Ksep400 system. The number of chambers used (and cycles applied) is dependent on the total cell density and volume of culture broth to be clarified.

Parameter	Value	Description / Remark
Chamber loading volume CV [mL/Ch]	1100[1]	Adjusted chamber load volume, targeting ~ 90% chamber fill with VPC
g-force [x g]	1000	Constant over complete process
Load flow rate [mL/min/Ch]	150	Load cell broth
Wash flow rate [mL/min/Ch]	150	Washing with buffer fluid
IDV [mL/Ch]	100	Initial dump volume, due to void volume of chamber
WV [mL/Ch]	260	Wash volume, until supernatant is completely elutriated
Cell dump flow rate [mL/Ch/min]	300	Discharge cells from the chamber(s)
Cell Dump Volume [mL/Ch]	150	
Used chambers	1 - 4	The number of chambers depends on the culture volume to be processed

[1] As a result of loading culture broth with ~ 6 x 10^6 total Viral Production Cells per mL

Ksep400 Protocol

The Ksep400 clarification process is started by priming the tubing set and the chambers with wash buffer (i.e., PBS) as well as by ramping-up the centrifugal G force. In the first cycle step, the chambers are loaded with cell culture broth and the supernatant is collected in a harvest container (product reservoir bag). After the chamber is loaded with a certain amount of biomass, the cells in the fluidized bed are washed with buffer, thereby further recovering LV vector product. Finally, cells are discharged from the chambers by reverting the flow direction. This automized, semi-continuous centrifugation procedure of sequential loading, washing, and discharging steps is repeated until a desired culture volume is clarified.

Ksep400 Process Parameter Considerations

For successful fluidized cell bed formation using the Gibco VPCs, a load of ~ 7 x 109 total cells per chamber resulted in the chamber being filled to 90% capacity when using the recommended process parameters (Table 2). It is observed that a filling level of approximately 90 % at the end of the loading step results in an almost cell-free clarified harvest7.

The pre-determined number of 7 x 109 total cells can be used to estimate the chamber loading volume (CV) of cell culture broth based on the cell densities observed at the time of harvest.

For the Gibco VPCs, an initial parameter screening was performed (at constant g/f ratio) and their effect on turbidity reduction and cell removal from the supernatant is shown in Figure 6 below. These parameters should be optimized to achieve high performance clarification for each cell production system.

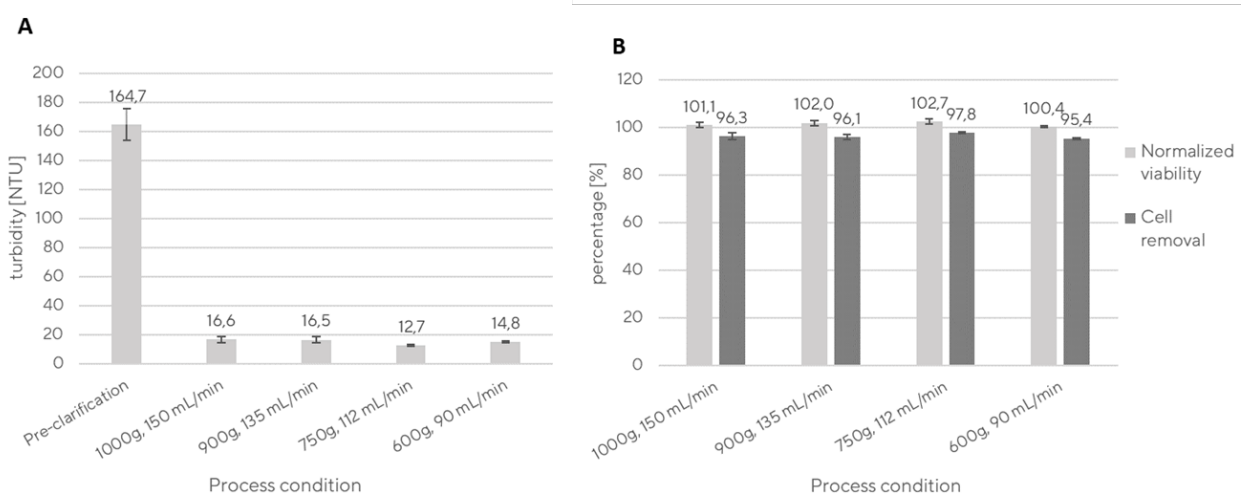

Figure 6: Ksep400 Fluidized Cell Bed Formation Parameters. *Centrifugal and fluid flow force parameter screening for Ksep400 (1000G, 150mL/min; 900G, 135mL/min; 750G, 112mL/min; and 600G, 90mL/min) yielded in A) high turbidity reduction and B) high normalized cell viability and >90% cell removal.*

This shows that a wide range of Ksep400 operating parameters work well for VPC processing. Considering process scale up, the condition with the highest flow rate and g-force (1000G, 150 mL/min) is recommended as it results in the overall shortest processing time (and was used for experimental runs for the Ksep400 as shown in the main blog article).

d) Analytical Methods

Titration of infectious lentiviral vector titer done according to the protocol described in the LV-MAX™ Lentiviral Production System User Guide. Turbidity measurements were carried out using a AQUAfast AQ3010 Turbidity Meter according to the manufacturer´s instructions