Lentiviral Vector Preparation Application Note

LENTIVIRAL VECTOR PREPARATION USING OPTIMA X SERIES ULTRACENTRIFUGES AND SW 32 TI ROTOR

Viral vectors are one of the most commonly used tools for genetic manipulation in mammalian cells. They are used both for gene expression as well as gene silencing. Lentiviruses are commonly used viral vectors due to their ability to infect both dividing and non-dividing cells, including stem cells. When infecting cells with these viral vectors, it is often necessary to concentrate virus particles to achieve high titer, especially when a large number of cells must be infected or for use with certain cell lines which are resistant to transduction. Centrifugation is a simple and effective method to concentrate viral particles. Here we present two simple methods of concentrating the viral vector. These methods are equally useful for concentrating viral particles for other studies, like nucleic acid sample prep or electron microscopy.

Concentration of Lentiviral particles using Polyethylene Glycol (PEG):

1. Collect your viral supernatant from transfected packaging cells and pass it through a sterile 0.45 µm filter to remove any loose cells and cell debris.
2. Mix the supernatant with 40% PEG solution to a final PEG concentration of 10%. Incubate the mixture on ice for 3 to 6 hours.
3. Spin in centrifuge at 2000 x *g* for 30 minutes.
4. Discard the supernatant. Disperse viral particles pellet by pipetting in 1/20 of the original harvest volume of PBS (Phosphate Buffered Saline) or the media of your choice.
5. To further concentrate, transfer the viral particles to pre-sterilized ultracentrifuge tubes.
6. Place the tubes into buckets. Weigh and balance them.
7. Spin at 100,000 x *g* (24,500 RPM) at 4°C in a SW 32 Ti rotor for 90 minutes, in a Beckman Optima X Series ultracentrifuge.
8. Remove the supernatant by inversion of the tubes or pipetting; be careful not to dislodge the viral pellet.
9. Re-suspend the pellet in PBS or the media of your choice.
10. Pipette up and down or shake for a few minutes, if necessary, to fully dissolve the pellet.
11. Aliquot and store at desired temperature; ultra-low temperature (ULT) storage is recommended for long term.

Concentration of Lentiviral particles using sucrose cushion:

1. Collect your viral supernatant from transfected packaging cells and pass it through a sterile 0.45 µm filter to remove any loose cells and cell debris.
2. Add 3–5 mL of 20% sucrose carefully to the bottom of pre-sterilized ultracentrifuge tubes.
3. Overlay viral supernatant carefully over the sucrose cushion.
4. Place the tubes into buckets. Weigh and balance them.
5. Centrifuge the supernatant at 125000 x *g* at 4°C in SW 32 Ti rotor for 90 minutes, in a Beckman Optima X Series ultracentrifuge.
6. Remove the supernatant by inversion of the tubes or pipetting; be careful not to dislodge the viral pellet.
7. Re-suspend the pellet in PBS or the media of your choice.
8. Pipette up and down or shake for a few minutes, if necessary, to fully dissolve the pellet.
9. Aliquot and store at desired temperature; ULT storage is recommended for long term.

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SW 32 TI ROTORS					
	Rotor Type	Max rpm/Max Force x *g*	Max Capacity	R Max	Part Number
SW 32 Ti	Swinging Bucket	32,000 rpm/175,000 x *g*	231 mL	152.5 mm	369694

COMMON TUBES AND ADAPTERS FOR SW 32 TI ROTOR		
Tube	Part Number	Adapter/Spacer
Tube, OptiSeal, Polyallomer, 32.4 mL, 25x77 mm	361625	392833
Tube, Quick-Seal, konical, Polyallomer, 28 mL, 25x83 mm	358651	355536 and 358156
Tube, Quick-Seal, konical, Polyallomer, 8.4 mL, 25x38 mm	358652	355536 and 358156
Tube, Quick-Seal, Polyallomer, 15 mL, 25x38 mm	343664	355536
Tube, Quick-Seal, Polyallomer, 27 mL, 25x64 mm	343665	355536
Tube, Quick-Seal, Polyallomer, 33.5 mL, 25x83 mm	344623	355536
Tube, Thickwall, Polyallomer, 31 mL, 25x89 mm	355642	—
Tube, Thickwall, Polycarbonate, 31 mL, 25x89 mm	355631	—
Tube, Thinwall, konical, Polyallomer, 25.5 mL, 25x76 mm	358125	358156
Tube, Thinwall, konical, Polyallomer, 31.5 mL, 25x89 mm	358126	358156
Tube, Thinwall, Polyallomer, 38.5 mL, 25x89 mm	326823	—
Tube, Thinwall, Ultra-Clear, 38.5 mL, 25x89 mm	344058	—

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B2013-14076-500-LC

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