Isolere Bio™
By Donaldson

IsoTag™ LV User Manual

ALPS-TFF

for LV Purification

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1. About This Manual

This manual is part of the product; it must be read in full and retained. This manual applies to the following versions of the product:

IsoTag™ LV, 10mL Evaluation Kit
IsoTag™ LV, 5mL Reagent (min. x2 required)

2. Intended Use

The product is intended for **research use only.** It is **not** for diagnostic use or direct administration to humans or animals. The product is intended exclusively for use in accordance with this manual. Any other use is considered improper.

3. Product Description

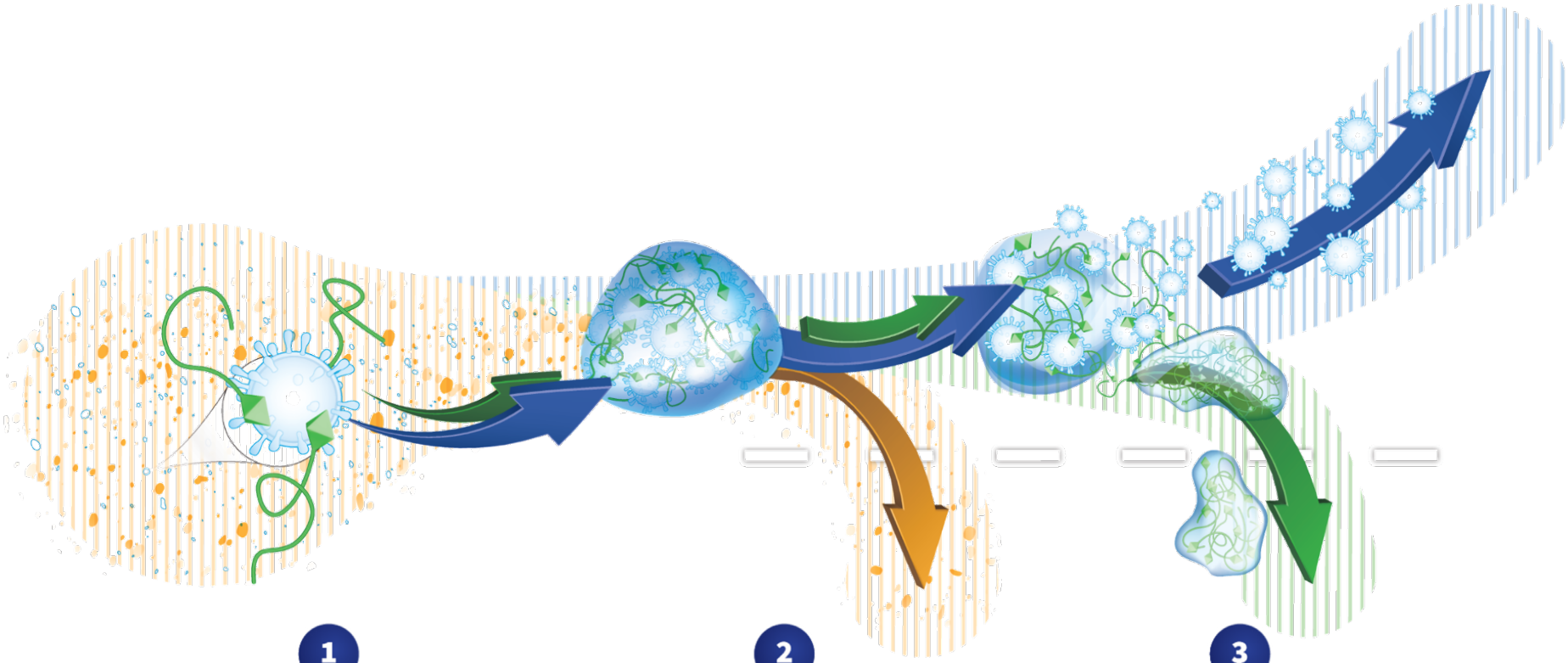
IsoTag™ LV combines the principles of affinity capture with liquid-liquid phase separation using a proprietary fusion protein. The single protein reagent has two domains: (1) an LV-specific binding domain and (2) a stimulus-responsive biopolymer.

IsoTag™ LV is a specialized reagent, engineered for the demanding requirements of small and large-scale downstream purification. IsoTag™ LV reagent enables an efficient purification process for VSV-G pseudotyped lentivirus (LV).

Features of IsoTag™ LV include:

- Efficient purification with high purity and yield
- Linear scalability based on culture volume, rather than LV titer
- Lower LV aggregation than traditional affinity chromatography
- Compatibility with existing, familiar TFF equipment and off-the-shelf consumables

4. Affinity Liquid Phase Separation Overview



1

Bind in solution

The binding domain targets a specific biomolecule of interest, binding in seconds (no mass transfer limits)

2

Concentrate & wash

Pull target into large droplets that are in a separate phase from other aqueous contaminants.

3

Final product

Efficiently collect high-yield product with filtration or low speed centrifugation: no new equipment or consumables (e.g. filters) needed.

5. Specifications

Characteristic	Description
Appearance	Clear, pale yellow or colorless liquid
Formulation buffer	0.25X PBS (pH 7.2 ± 0.2)
Concentration*	9.4mg/mL (+20%, -0%)
Pseudotype affinity	VSV-G
Recommended concentration for use	3.6% v/v for titers below $1.0x10^9$ TU/mL
Buffer additives	PBS and water. *The use of urea may cause inhibition of the phase behavior. Addition of EDTA will inhibit affinity activity of the reagent.*
Storage conditions	-80˚C until use. *Allow material to acclimate at -80˚C for ≥24 hours once removed from dry ice.*

**For specific IsoTag™ LV lot concentration, see the Certificate of Analysis*

6. Method

The following method is intended for use with 250mL of LV harvest material.
All equipment, reagent and buffer volumes have been specified for 250mL of LV harvest material.

6.1 Equipment

- Lab scale TFF system with Masterflex L/S tubing size 14, or similar setup
- Two auxiliary pumps
- HFF 1 (Hollow fiber TFF filter for first stage): pore size of 0.65 µm and total surface area between 50-150 cm^2
- HFF 2 (Hollow fiber TFF filter for second stage): pore size of 0.05 µm and total surface area between 50-200 cm^2
- Small stir plate
- Tubing clamps (x3)
- Y- or T-fittings (x3)
- Ice bucket or cold pack

- 0.22 µm bottle top filters (x5)
- Rocker
- Conical bottom retentate vessel that can accommodate working volumes of 20 - 150mL with at least 4 inlet and outlet tubing ports (x2)
- Recommended: universal clamp stand
- Recommended: one-way valves (x2-3)

6.2 Materials

Description	Composition	Volume Required
IsoTag™ LV Reagent	IsoTag™ LV, 0.25X PBS (pH 7.2 ± 0.2)	9.1 mL
Phase Transition Buffer Concentrate*	5.25M NaCl, 3.9mM $CaCl_2$	75 mL
DI Water*	DI Water	150 mL
Equilibration Buffer*	20mM Tris, 1.2M NaCl, 0.9mM $CaCl_2$ pH 7.0-7.5	50 mL
Wash Buffer*	20mM Tris, 1.2M NaCl, 0.9mM $CaCl_2$ pH 7.0-7.5	100 mL
Elution Buffer*†	200mM Arginine, 100mM Glutamic Acid, 20mM Tris, pH 6.5	130 mL
Storage Buffer*	(suggested) 150mM NaCl, 50mM Tris, pH 7.5	100 mL

**Buffers not supplied.*

†*Conductivity target for elution buffer is 29.3 - 31.1 mS/cm*

6.3 Day 0: Buffer Preparation

1. Prepare all buffers at the minimum volume required in section 6.2 and filter through 0.22 µm bottle top filter.
2. Store the Elution and Storage Buffers at 4°C overnight.

6.4 Day 0-1: Treatment of LV Harvest Material

This step ensures the IsoTag™ LV has adequate contact time to bind to the LV particles – mixing is critical to TFF1 success.

1. Start with fresh or thawed, nuclease-treated clarified LV harvest material.
2. Thaw IsoTag™ LV reagent on ice.
3. Add IsoTag™ LV reagent to the clarified harvest at a working concentration of 3.6% v/v and incubate harvest-IsoTag™ LV mixture together overnight at 4˚C while gently mixing

on a rocker.

4. **Day 1:** Add Phase Transition Buffer Concentrate to the clarified harvest at a working concentration of 1.2M NaCl (74.3 mL for a 325 mL total volume) and mix. Bring the harvest, IsoTag™ LV, and NaCl mixture to room temperature (18-22˚C).

Optional: place in water bath to speed up warming to room temperature.

5. Once at room temperature, mix for a minimum of 20 minutes and a maximum of 60 minutes to allow droplet formation. Consider this material as “treated harvest”.

6.5 Day 1: System Preparation

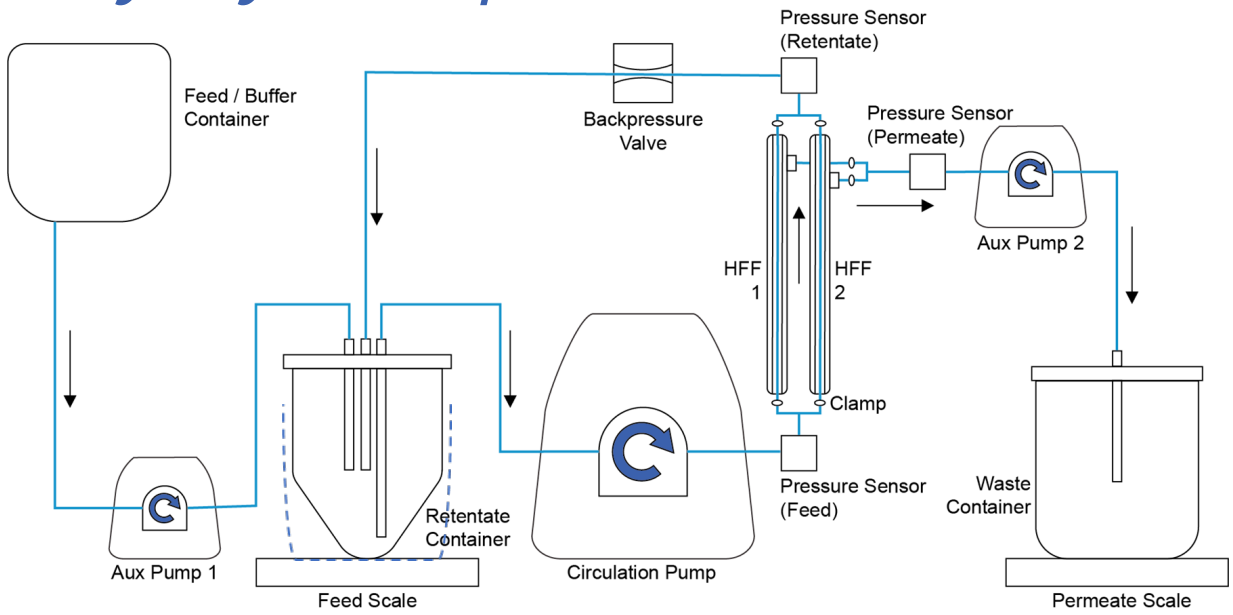


Figure 1 - Diagram of two-stage TFF setup. Blue dotted secondary container for ice used during Elution steps.

1. Set up the equipment as outlined in Figure 1. Clamps are required and one-way valves are recommended at all HFF connection points. Aux pump 2 is required for permeate control mode necessary for microfiltration.

2. Place a second retentate vessel on ice.

3. In Manual mode pump 150 mL DI water into the Retentate Container through aux pump 1 and flush through system with all lines unclamped with a Circulation pump flow rate of 50 mL/min.

4. Once the Retentate Container is nearly empty, stop the Circulation pump and clamp HFF 2 at all connection points. Avoid introducing air into the system by allowing the container to completely empty.

5. Pump 50mL of Equilibration Buffer into Retentate Container through aux pump 1 and flush through the system, ensuring the Equilibration Buffer is only flushed through HFF 1. Be careful not to introduce air into the system during buffer changeover.

6. Once the Retentate Container is nearly empty, close the permeate line and stop the Circulation pump.
7. Empty waste container. Tare the Feed and Permeate scales.

6.6 Day 1: Concentration and Wash of LV Material

In this step, concentrate the treated LV harvest 12.5X, or to a 20mL volume and diafilter with Wash Buffer (5 DVs) to concentrate and wash the treated harvest.

1. Connect the diafiltration line to the vessel containing the treated harvest (Feed Container). Be careful not to introduce air into the system. Using aux pump 1, pump 20mL of the treated harvest into the Retentate Container.
2. In Manual mode, set the crossflow rate to achieve a shear rate of 2000-2500 sec^{-1} (example: About 105 mL/min for a filter area of 85 cm^2). Recirculate the retentate for 15-20 minutes prior to starting the run.
3. Set up an automated C/D recipe (concentration/diafiltration) with a crossflow rate set to achieve a shear rate of 2000-2500 sec^{-1}. Set the concentration factor (CF) to 1 and diafiltration value (DV) to 22. Set the backpressure valve to a retentate pressure of 10 psi.
4. Start the run with a crossflow rate to achieve a 2000-2500 sec^{-1} and the permeate flow closed (aux pump 2 paused), if possible, or set to the minimum possible flow rate until the retentate pressure reaches the 10 psi set point and remains steady.
5. Ensure aux pump 2 (permeate pump) speed is set to 2 mL/min and start pump.
6. Ramp up aux pump 2 flow rate by 0.5 mL/min, every 3-5 minutes, up to a maximum flux value of 50 LMH.
   NOTE: if the TMP begins to rise significantly while ramping up the aux pump 2 flow rate, do not continue to increase the flow rate.
7. Collect flow-through fractions on the permeate side (Waste Container) of the HFF1 for analytics as desired.
8. Immediately after the Feed Container has been emptied, add 100mL of Wash Buffer into the Feed Container or transfer the Diafiltration inlet tubing into a Secondary Reservoir containing Wash Buffer. Be careful not to introduce air into the system during buffer changeover.
9. Continue with 5 DVs of Wash Buffer wash.
10. Collect wash fractions and samples for analytics as desired.
11. Close the permeate line and stop the Circulation pump when 100 mL of Wash Buffer has been consumed.

6.7 Day 1: HFF1 Flush

This step is critical to maximizing LV recovery. LV bound to IsoTag™ LV on HFF1 can be removed with a cold Elution Buffer flush through both the retentate and permeate and recovered for elution.

1. Remove the Retentate Container containing the concentrated LV from the system and place on ice. Replace with the second Retentate Container from section 6.5. Keep the second Retentate Container submerged in ice or wrapped in ice packs for the duration of section 6.7 and 6.8. Maintaining a cool temperature of the retentate will help LV stability, IsoTag reagent clearance and efficient filter utilization.
   NOTE: the concentrated LV will be returned to the system during Step 7.
2. Tare the scales.
3. Attach permeate line to an inlet port on the Retentate Container and ensure it is clamped closed.
4. Switch the system to Manual mode to perform a flush of HFF1 with Elution Buffer. This is critical to the recovery of LV and IsoTag™ LV on the filter.
5. Pump 130 mL of 4°C Elution Buffer into the Retentate Container through aux pump 1 and start the Circulation pump. Recirculate the buffer at 90 mL/min for 10 minutes through HFF1.
6. After 10 minutes, open the permeate line (aux pump 2) and allow the Elution Buffer to flow through the permeate side of HFF1 and back into the Retentate Container for 10 minutes.
7. After the 20-minute total flush, stop the Circulation pump and return the permeate tubing line to waste. Return the concentrated LV into the new Retentate Container using aux pump 1. Mix and take a sample for analytics as this represents the captured and concentrated LV.

6.8 Day 1: Elution of purified LV

In the next two steps, remove IsoTag™ LV through a series of dilutions and concentrations on the smaller pore HFF2.

1. Remove the clamps for HFF2 and clamp HFF1 so that the solution only passes through HFF2.
2. Recirculate the retentate for 5-10 minutes at 90 mL/min prior to starting the run with permeate line closed.
3. In Manual mode, set the crossflow rate to achieve a shear rate of 2000-2500 sec^{-1}

(example: About 90 mL/min for a filter area of 150 cm^2). Set the backpressure valve to a retentate pressure of 10 psi.

4. Start the Circulation pump and keep the permeate flow closed (aux pump 2 paused), if possible, or set to the minimum possible flow rate until the retentate pressure reaches the 10 psi set point and remains steady. Ensure aux pump 2 (permeate pump) speed is set to 2 mL/min and start pump.

5. Ramp up aux pump 2 flow rate by 0.5 mL/min, every 3-5 minutes, up to a maximum flux value of 50 LMH, likely ending around 5 mL/min.
   NOTE: if the TMP begins to rise significantly while ramping up the aux pump 2 flow rate, do not continue to increase the flow rate.

6. Concentrate 15X, or to a final volume of 10 mL.

6.9 Day 1: Final Buffer Exchange of LV

This step continues from section 6.8 to buffer exchange the purified LV into a Storage or Formulation Buffer while continuing to remove IsoTag™ LV.

1. Once the concentration factor is reached (target final volume of 10mL), stop aux pump 2 but keep the Circulation pump running.

2. Dilute the retained LV 1:10 by pumping 4°C Storage Buffer into the Retentate Container using aux pump 1 up to a total volume of 100 mL.

3. Start aux pump 2 and concentrate to a suggested final volume of 10 mL. This will culminate to a 25X concentration from starting harvest material volume. Additional concentration is possible but unoptimized.

4. Collect the purified LV from the Retentate Container and store at –80˚C or use immediately.

7. Analytical Interference

High NaCl concentration

Impacted Assay	Suggested alteration
p24 ELISA	Dilute samples prior to use. Include extra controls for high NaCl interference.
qRT-PCR	Dilute samples prior to use. Include extra controls for high NaCl interference.
FACS	Dilute samples prior to use. Include extra controls for high NaCl interference.

IsoTag™ LV

Impacted Assay	Suggested alteration
p24 ELISA	Dilute samples prior to use. Include extra controls for IsoTag™ LV interference.
qRT-PCR	Dilute samples prior to use. Include extra controls for IsoTag™ LV interference.
FACS	Add 15 µg/mL of Polybrene into cell culture with analytical samples. Include controls with and without Polybrene and IsoTag™ LV. Other transduction-enhancing products may require additional optimization.
VSV-G Western Blot	Use 5X the recommended reducing agent.

8. Process Optimization

Process Step	Optimization
Harvest material treatment	The method described in this document uses harvest material that was clarified and treated to remove cellular debris and DNA. Depending on the feedstream used, it may be possible to remove the pre-treatment. Further development may be needed for each feedstream.
TFF equipment	The method described in this document was optimized for the Kr2i TFF system. When using other systems, the user should ensure that the shear rate during the concentration process is below 2500 sec^{-1} to maintain LV stability. Increasing the flux as described in the recommended protocol should also proceed at a slow rate to prevent fouling of the membrane.

Process Step	Optimization
Incubation time	The method described in this document strongly recommends an overnight incubation with the harvest material and IsoTag™ LV reagent mixing at 4˚C. Altering the incubation time may be necessary to optimize binding of IsoTag™ LV to specific feed streams. Incubation time may be decreased with additional testing.
Recirculation with HFF1	The method described in this document is not optimized specifically for the users harvest material. Increasing recirculation time prior to the concentration and wash step may be needed to ensure maximum recovery during the concentration step. Likewise, decreasing recirculation time is possible to reduce total time. Adding a higher concentration of IsoTag™ LV to the initial solution in the retentate container (2.4 – 4 mL) could also reduce recirculation time requirements.
Wash	The method described in this document is not optimized specifically for the users harvest material. Altering the concentration of Phase Transition buffer may be necessary to optimize lentiviral recovery. Recommended concentration of NaCl is 0.6-1.2 M.
Elution	The method described in this document is not optimized specifically for the users harvest material. Increasing the contact time with the Elution Buffer and/or including multiple rounds of dilution in Elution Buffer could improve removal of IsoTag™ LV. Likewise, each user is recommended to optimize the components of the elution buffer for their specific feed stream.
LV pseudotype compatibility	The method described in this document was optimized for VSV-G pseudotyped LV. Additional optimization may be needed for other VSV-G pseudotyped particles.

9. Troubleshooting

Troubleshooting: **Initial Harvest Treatment and Preparation**

Observation	Possible Cause	Recommended Action
Harvest material is not cloudy after IsoTag™ addition	• Incorrect temperature • Incorrect salt concentration • Incorrect IsoTag™ LV concentration • Phase transition buffer is not prepared to the correct conductivity • Additive in cell culture interferes with phase behavior	• Allow sufficient time to allow the material to stabilize to room temperature. • Confirm transition of IsoTag™ LV in saline solution at similar dilution and salt concentration. • Confirm conductivity of all solutions, for 5.25 M NaCl, dilute to 1 M before reading conductivity. • Remove EDTA, urea, and detergents from cell culture or contact customer support

Troubleshooting: **Capture Step**

Observation	Possible Cause	Recommended Action
TFF1 waste is hazy	• Incorrect temperature • Incorrect salt concentration • Incorrect IsoTag™ LV concentration • Phase transition buffer is not prepared to the correct conductivity • Incorrect filter pore size is used	• Recirculate the permeate waste into the feed to improve product recovery and “clear” the waste product. Increase IsoTag™ LV and salt content as necessary. • Confirm transition of IsoTag™ LV in saline solution at similar dilution and salt concentration • Confirm conductivity of all solutions • Confirm correct filter is used

Observation	Possible Cause	Recommended Action
Fouling of TFF filter	• Contaminant profile of harvest material • Running outside suggested shear rate range • Running outside suggested flux (LMH) range • Incorrect retentate pressure	• Consider additional clarification or nuclease treatment • Reduce filter loading on a volume to meter squared basis • Confirm TFF run settings, consider running at a lower flux or ramping the flux at a slower rate • Calibrate pumps and replace pressure sensors
Incomplete capture, loss of material.	• Incorrect IsoTag™ LV concentration for specific harvest • Presence of harvest material additives • Incorrect filter pore size	• Increase IsoTag™ LV working concentration • Confirm absence of EDTA in harvest fraction • Consider diluting LV harvest 1:1 before capture
Pressure spikes during TFF1	• Fouling of TFF filter • Insufficient harvest material clarification	• Consider additional clarification or nuclease treatment

Troubleshooting: **Elution Step**

Observation	Possible Cause	Recommended Action
Retentate does not turn clear on addition of elution buffer	• Elution buffer temperature is too high • Elution buffer is at the wrong conductivity	• Ensure elution buffer is thoroughly cooled and is at 4°C prior to use • Place ice or a cold pack around the retentate vessel.

10. Order Information

Item Number	Description
100003076	IsoTag™ LV, 5 mL
100003078	IsoTag™ LV, 10 mL Evaluation Kit

For more information, please contact us at IsolereSupport@donaldson.com

11. Support

For technical support or to obtain a Certificate of Analysis, please contact us at IsolereSupport@donaldson.com

12. Limited Product Warranty

Isolere Bio, Inc and/or its affiliate(s) warrant their products as set forth in the Isolere Bio General Terms and Conditions of Sale found on Isolere Bio's website at www.isolerebio.com/terms-and-conditions-of-sale

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