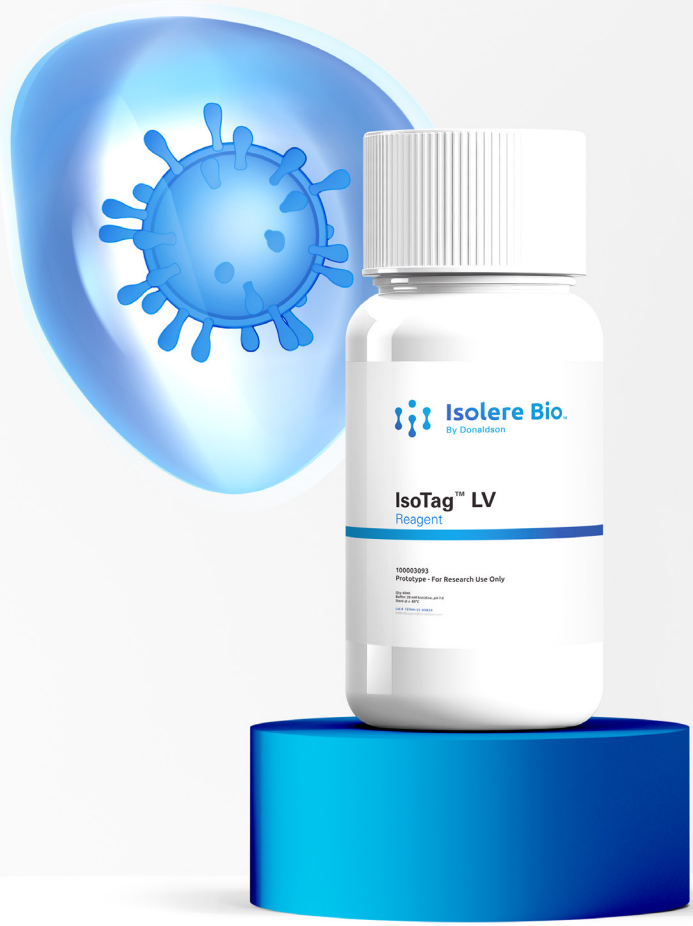
Isolere Bio™
By Donaldson

IsoTag™ LV User Manual

ALPS-CF



for LV Purification

Table of Contents

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, 5mL Evaluation Kit
IsoTag™ LV, 5mL Reagent

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. It enables a robust, efficient, and consistent purification process for VSV-G pseudotyped lentivirus (LV).

Features of IsoTag™ LV include:

- Single-step 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 centrifugation equipment and off-the-shelf consumables

4. Affinity Liquid Phase Separation Overview

5. Specifications

Characteristic	Description
Appearance	Clear, pale yellow or colorless, liquid
Formulation buffer	0.25X PBS, pH 7.0
Concentration	9.4 mg/mL (+20%, -0%)
Pseudotype affinity	VSV-G
Recommended concentration for use	0.19 mg/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.*

6. Method

The following method is intended for use with 200mL of LV harvest material.
All equipment, reagent and buffer volumes have been specified for 200mL of LV harvest material.

For different purification volumes, see the chart below for guidance on material and resource usage.

Crude Lentivirus Volume (mL)	IsoTag™ LV (mL)	Phase Transition Buffer (mL)	Optional Wash Buffer (mL)
200	5	45	40
100	2.5	23	20
40	1	9	8
5	0.13	1.2	1

6.1 Equipment

- Benchtop centrifuge
- Water bath or heat block
- Tube rotator

6.2 Materials

Component	Composition
IsoTag™ LV Reagent	IsoTag™ LV, 0.25x PBS, pH 7.0
Phase Transition Buffer*	5.5M NaCl, 4.5mM $CaCl_2$
Wash Buffer Concentrate*	500mM Tris

**buffers not supplied*

6.3 Sample Preparation

1. Thaw IsoTag™ LV on ice.
2. *Optional: nuclease treat and clarify LV material.*
3. Warm water bath to 37°C
4. *Recommended: warm centrifuge to 37°C*

6.4 Lentiviral Capture and Concentration

1. Add crude LV to an appropiately sized container and place on ice.
2. Add the appropiate volume of IsoTag™ LV from the table (0.19 mg/mL) and mix by inversion or swirling and rest on ice for 1 hour. A 1-hour incubation on ice before salt addition is necessary for successful binding between IsoTag™ LV and LV material.
3. Add Phase Transition Buffer according to the table (to a final concentration of 1M) and invert or swirl to mix.
4. Place the container in the water bath set to 37°C for 10 minutes to allow droplet formation. Mix regularly. The solution should quickly become cloudy. If the solution is not cloudy after 10 minutes, continue incubation for an additional 10 minutes with

regular mixing. Avoid foaming/bubbles while mixing.

Recommended: Use thermomixer set to 600 rpm and 37°C to mix samples.

5. If necessary, separate mixture into tubes compatible with centrifuge.
6. Centrifuge for 10 minutes at 2500 g at 37°C. The capture pellet should be visible after centrifugation. The capture pellet contains the LV material bound to IsoTag™ LV.
7. Remove the supernatant without disturbing the pellet.

Optional: save the capture supernatant in a separate conical for analysis.

NOTE: If proceeding to Optional Wash (6.5), please skip next step (6.4.8) below.

8. Pellets can be resuspended by rotation at 4°C in volume and buffer of choice, pooled, and used immediately or stored at -80°C for future use.

6.5 Optional Wash

1. Prepare 50 mL Wash Buffer by combining 2 mL wash buffer concentrate with 9 mL Phase Transition Buffer and 39 mL DI water.
2. Following capture, immediately resuspend by rotation at 4°C and pool pellets in Wash Buffer to a total volume of half the total starting volume.
3. Place the sample in the water bath set to 37°C for 10 minutes to allow droplet formation. The solution should be cloudy. It may take longer (15-30 minutes) for larger volumes.
4. Centrifuge for 10 minutes at 2500 g at 37°C. The pellet should be visible after centrifugation. The pellet contains the LV material bound to IsoTag™ LV.
5. Remove the supernatant without disturbing the pellet.

Optional: save the supernatant in a separate conical for analysis.

6. Pellets can be resuspended in volume and buffer of choice, pooled, and used immediately or stored at -80°C for future use.

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 ug/mL of Polybrene into cell culture with analytical samples. Include extra controls for Polybrene and IsoTag™ LV interference. 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 centrifuged to remove cell debris. Depending on the feedstock used, it may be necessary to add a clarification step to maximize lentiviral recoveries. Further development may be needed for each feedstock.
Capture and concentration	The method described in this document was tested on a variety of feed streams of different titer. Depending on the feedstock used, it may be necessary to alter the IsoTag™ LV concentration to maximize lentiviral recovery.

9. Troubleshooting

Observation	Possible Cause	Recommended Action
Capture reaction is not cloudy after water bath incubation at 37°C	• Water bath is not at correct temperature • Incorrect salt concentration • Incorrect IsoTag™ LV concentration • Phase transition buffer is not prepared to the correct conductivity	• Confirm IsoTag™ LV in saline solution at similar dilution and salt concentration • Confirm conductivity of all solutions
Capture pellet is not visible after the first centrifugation step.	• Centrifuge is not at correct temperature • Incorrect salt concentration • Incorrect IsoTag™ LV concentration	• Confirm centrifuge is warmed to 37°C • Confirm centrifuge spin parameters are correct • Confirm conductivity of all solutions
Incomplete capture, loss of material	• IsoTag™ LV did not bind to LV material • Presence of harvest material additives • pH of harvest	• Increase IsoTag™ LV working concentration to 2X-5X amount recommended in protocol • Confirm absense of buffers containing Urea, Tris, Antifoam, EDTA, histidine • Confirm harvest material is above pH 5 and below pH 9

10. Order Information

Item Number	Description
100003076	IsoTag™ LV, 5mL
100003077	IsoTag™ LV, 5mL 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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