Isolere Bio™
By Donaldson

IsoTag™ AAV User Manual

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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™ AAV, 6.6mL Evaluation Kit
IsoTag™ AAV, 6.6mL 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™ AAV 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 AAV-specific binding domain and (2) a stimulus-responsive biopolymer.

IsoTag™ AAV 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 a broad spectrum of adeno-associated virus (AAV) serotypes.

Features of IsoTag™ AAV include:

- Single-step purification with high purity and yield
- Linear scalability based on culture volume, rather than AAV titer
- Lower AAV 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, colorless, liquid
Formulation buffer	20mM histidine, pH 7.0
Concentration	11.3 mg/mL (+20%, -0%)
Serotype affinity*	AAV9, AAV8
Recommended concentration for use	0.67 mg/mL in seed volume, 0.045 mg/mL in harvest material for AAV9
Buffer additives	Histidine, 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

**Binding efficacy observed with small-scale capture tests with AAV9, 8, 6, 2, 1, PHP.B and Rhs.10 – see IsoTag™ AAV white paper for more info (www.isolerebio.com/aavpaper)*

6. Method

The following method is intended for use with 1L of AAV9 harvest material. All equipment, reagent and buffer volumes have been specified for 1L of AAV9 harvest material.

6.1 Equipment

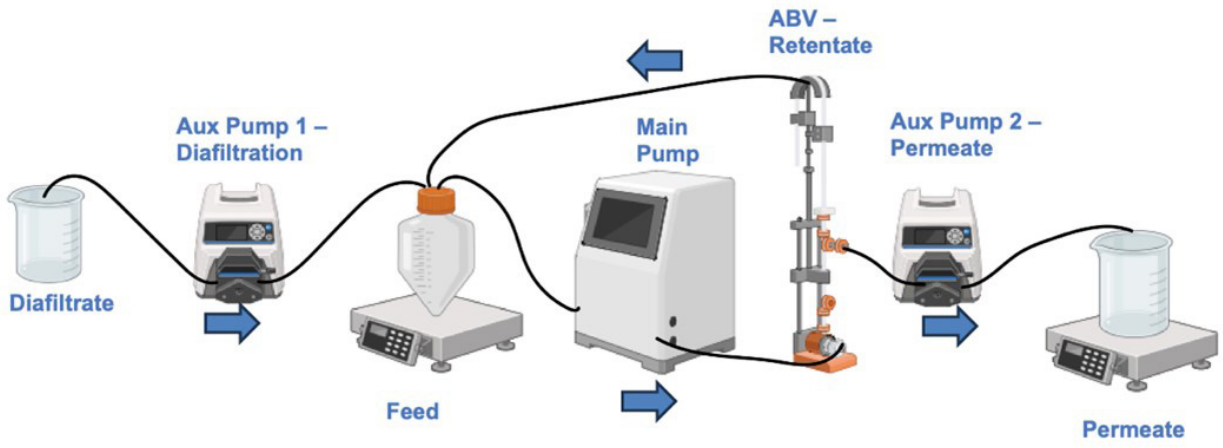
- Lab scale TFF system with *two auxiliary pumps*
- mPES hollow fiber filter - 0.2µm pore size, 140cm^2 surface area, 0.5mm fiber ID
- L/S 16 tubing (recommended Masterflex PharmaPure MFLX06435-16)
- L/S 14 tubing (recommended Masterflex PharmaPure MFLX06435-14)
- Retentate vessel that can accommodate working volumes of 20 - 120mL (Recommended bottom fed e.g. Pendotech PDKT-TNK125M)
- 0.2µm filter
- Recommended: small stir plate and stir bar for retentate vessel

6.2 Materials

Kit Component	Composition
IsoTag™ AAV Reagent	IsoTag™ AAV, 20mM histidine, pH 7.0
Phase Transition Buffer 1	5M NaCl
Wash Buffer	20mM Tris, 0.6M NaCl, 0.01% pluronic F-68, pH 7.5
Elution Buffer	100mM glycine, 0.01% pluronic F-68, pH 3.0
Phase Transition Buffer 2	5M $MgCl_2$
Neutralization Buffer	1M Tris-HCl, pH 7.5

6.3 System Preparation

1. Aliquot 80mL of Elution Buffer and place at 4˚C until elution step
2. Combine 100mL of Elution Buffer and 13.6mL of Phase Transition Buffer 2 into one container and set aside at room temperature.
3. Setup the TFF system
   - Place retentate vessel (with stir bar) and stir plate on feed scale
   - Connect main hold-up loop with L/S 16 tubing
   - Connect diafiltration line to retentate vessel with first auxiliary pump
   - Connect L/S 14 tubing to permeate side with second auxiliary pump
4. Prepare the TFF by equilibrating the system, pumping a minimum of 40mL of Wash Buffer through the retentate and permeate lines to fully prime and send to waste.



6.4 Treatment of AAV Harvest Material

1. Lyse, nuclease treat and clarify 1L AAV harvest material prior to IsoTag™ AAV purification.
2. Add 140mL of Phase Transition Buffer 1 and mix. Filter solution through 0.2µm filter to clarify material.
3. Aliquot 20mL of AAV material and add 1.2mL IsoTag™ AAV to achieve a 0.67mg/mL concentration in the seed volume. Incubate for 20 minutes to allow droplet formation.
4. To the remaining 1120mL of AAV material, add 4.52mL IsoTag™ AAV to achieve a 0.045mg/mL concentration in the bulk harvest material.
5. Incubate seed and feed AAV material with IsoTag™ AAV for at least 20 minutes at room temperature prior to beginning the run.

6.5 Concentration and Wash of AAV Material

1. Prime the diafiltration line with feed from the bulk harvest placed in the diafiltration buffer reservoir.
2. Connect diafiltration line to retentate vessel and tare feed scale.
3. Transfer the 20mL seed volume to the feed reservoir and set stirring speed to 400rpm.
4. If using Repligen software, change the program to C/D mode. Set the concentration factor (CF) to 1 and diafiltration value (DV) to 60. For general TFF settings, target a concentration factor of 56X.
5. Run the process in permeate control mode with the cross flow set to 265mL/min and the automated back pressure valve (ABV) set to control retentate pressure at 10psi.
6. Start the run with permeate flow set to minimum speed until the retentate pressure reaches the 10psi setpoint.
7. Ramp up the permeate speed by increasing 0.5mL/min every 3-5 minutes to ensure TMP stabilizes between each increase until the flux reach 45-55 LMH. Concentrate the retentate 56X, or to 20mL using fed batch process.
8. Upon completion of the feed and achievement of a 56X concentration factor, switch the diafiltration line to a reservoir containing 150mL Wash Buffer ensuring minimal bubbles are introduced into the system (run can be paused if necessary). Wash the retentate with Wash Buffer for 6 diafiltration volumes of 20mL for each diavolume.

6.6 Elution of Purified AAV Material

1. Switch the system to manual mode, close the permeate line, and open the back pressure valve.
2. Add 80mL of cold Elution Buffer to the feed reservoir. Recirculate the buffer at 265mL/min for 5 minutes.
3. Add 13.6mL Phase Transition Buffer 2 to the feed reservoir and continue recirculation of the material for an additional 10-15 minutes, allowing the solution to warm up to room temperature.
4. Prime the diafiltration line with the room temperature solution containing Elution Buffer and Phase Transition Buffer 2 aliquoted in step 6.3.
5. Once the solution has reached room temperature, stop recirculation, open the permeate line and place in new reservoir on permeate scale to collect product.

   (Note: the total elution volume will be ~200mL. It is recommended to collect elution concentrate (~100mL) and elution DVs (8x ~12mL) separately for analytical purposes.)
6. Start the run for a 10X concentration factor, allowing the retentate pressure to reach 10psi before ramping permeate flow rate to desired flux.

   (Note: elution step can be ramped quicker and run at higher flux than the concentration/wash).
7. Once the concentration factor is reached, proceed with the diafiltration step using 8 diavolumes of the solution containing Elution Buffer and Phase Transition Buffer 2.
8. To each permeate fraction, add 10% volume of Neutralization Buffer to neutralize the sample.

   (Note: it may be neccessary to buffer exchange elution samples prior to analytics. The conductivity of the elution sample has been seen to interfere with ddPCR titering).

7. 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 feedstock used, it may be possible to remove the pre-treatment. Further development may be needed for each feedstock.
Concentration and Wash	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 8000 sec^{-1} to prevent fouling of the membrane. Increasing the flux as described in the recommended protocol should also proceed at a slow rate to prevent fouling of the membrane.
AAV Serotype Compatibility	The method described in this document was optimized for AAV9. For other serotypes, increasing the concentration of IsoTag™ AAV reagent to 0.09mg/mL in the bulk material and 0.9mg/mL in the seed volume, or higher concentrations, may be necessary for optimal capture and purification.
Elution	Elution buffer optimization may be neccessary. The following buffers are recommended. • 100mM Glycine, pH 3.0 • 0.5M Arginine, pH 7.0 • 0.5M Proline, pH 2.5

8. Troubleshooting

Troubleshooting: Initial Harvest Treatment and Preparation

Observation	Possible Cause	Recommended Action
Harvest material is not cloudy after IsoTag™ AAV addition	• Incorrect temperature • Incorrect salt concentration • Incorrect IsoTag™ AAV concentration • Phase transition buffer is not prepared to the correct conductivity	• Confirm transition in saline solution at similar dilution and salt concentration • Confirm conductivity of all solutions

Troubleshooting: Capture Step

Observation	Possible Cause	Recommended Action
IsoTag™ AAV permeate is not clear	• Incorrect temperature • Incorrect salt concentration • Incorrect IsoTag™ AAV concentration • Phase transition buffer is not prepared to the correct conductivity • Incorrect filter pore size is used	• Confirm transition in saline solution at similar dilution and salt concentration • Confirm conductivity of all solutions • Confirm correct filter is used
Fouling of TFF filter	• Process not run in permeate control mode • Contaminant profile of harvest material • Running at the wrong shear rate • Incorrect flux (LMH) • Incorrect retentate pressue • Incorrect size/volume ratio	• 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 lower rate • Calibrate pumps and replace pressure sensors
Incomplete capture, loss of material.	• Incorrect IsoTag™ AAV concentration • Presence of harvest material additives • pH of harvest is incorrect	• Increase IsoTag™ AAV working concentration to 2X amount recommended in protocol • Confirm absense of EDTA in harvest fraction
Pressure spikes during TFF run	• 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 • Ensure no salt has been added to Elution Buffer
Retentate does not turn cloudy on addition of Phase Transition Buffer 2	• Insuffcient amount of Phase Transition Buffer 2 is added • Retentate temperature is too low	• Confirm correct buffer and volume is added • Ensure retentate is allowed to warm to room temperature

9. Order Information

Market Number	Description
100003069	IsoTag™ AAV, 6.6mL
100003070	IsoTag™ AAV, 6.6mL Evaluation Kit

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

10. Support

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

11. 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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