Thermo Scientific Aspire Protein A Chromatography Tips

User Manual

Thermo Scientific Aspire Protein A Tips

Catalog number	Description
990-03	**Thermo Scientific Aspire Protein A Tips** (96 purifications)

Introduction

Thermo Scientific Aspire Protein A Tips facilitate small-scale purification of multiple subclasses of IgG from serum, ascites and cell culture supernatants in minutes. The Aspire tip is a 1000µl pipette tip containing 150µl of Thermo Scientific Pierce Immobilized Protein A Plus resin slurry (50µl resin bed) with the capacity to purify ≥ 1.0mg of human IgG. The Aspire tips offer a fast and easy-to-employ purification protocol without compromising purity and yield.

Protein A is a bacterial cell wall protein produced by various strains of *Staphylococcus aureus* and binds to mammalian IgGs through interactions with Fc regions. The affinity between Protein A and IgG is not equivalent for all species, and the binding characteristics might differ amongst some subgroups of IgG within a species. The Aspire Protein A Tips coupled with the pre-optimized buffer system exhibit excellent binding characteristics resulting in high purity and yield.

The unique color-coded, multichannel Aspire protocol is optimized for low to medium throughput IgG purification without the need for centrifugation. This kit contains sufficient reagents to purify IgG from 96 individual samples. The Aspire tips are compatible with a variety of both single and multichannel pipettes, including the Thermo Scientific Finnpipette Novus (100-1200µl) and Gilson P1000 Pipetman. Up to eight samples can be processed in parallel on a multichannel pipette in less than 20 minutes.

Kit Contents

- **Aspire Protein A Tips (96 tips)**
- **Protein A IgG Binding Buffer**
- **Protein A IgG Wash Buffer**
- **200X Sample Dil. Buffer (Emulsion)**
- **IgG Elution Buffer**
- **IgG Neutralization Buffer**
- **Assorted colored tubes**
- **Micro-titer tube rack**

Storage

Upon receipt, store buffers and Aspire tip rack at 4°C. All other kit components can be stored at room temperature. Product is shipped at ambient temperature.

Procedure Using a Handheld Pipette

A. Additional Materials Required

- 1000µl single or multichannel pipette (manual or electronic)

B. Material Preparation

1. **1X Sample Dilution Buffer**: Dilute 200X Sample Dilution Buffer to 1X in Protein A IgG Binding Buffer. Vortex to mix. The total volume needed is determined by the quantity of tips being used and the volume of sample being processed per tip.
   Note: This mixture should be prepared fresh prior to sample prep.

2. **Sample Dilution:** Dilute the sample a minimum of 1:1 in 1X Sample Dilution Buffer (prepared in step 1) to a total volume of 250µl per fraction.
   For example: Add 125µl of sample to 125µl of 1X Sample Dilution Buffer or add 25µl of sample to 225µl of 1X Sample Dilution Buffer.
   Note: Each tip has the capacity to purify 1mg of human IgG. Diluted sample volumes exceeding 250µl must be processed using multiple diluted sample fractions. Additional yellow tubes are provided to prepare two diluted sample fractions per tip.

C. Purification Protocol

Note: Procedure can be performed at room temperature or 4°C.

1. Arrange the colored tubes in the provided rack as illustrated in diagram 1. Each sample requires the following quantities of colored tubes for processing:
   - 1 blue
   - 1 yellow
   - 3 green
   - 2 orange

   Note: Up to 8 samples can be processed in parallel using a multichannel pipette.

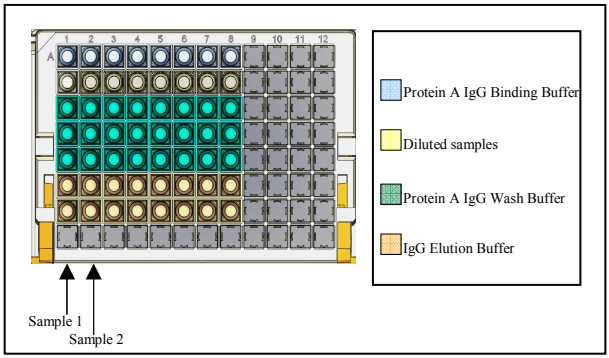


Diagram 1. Tube arrangement for processing eight samples

2. Aliquot 250µl of Protein A IgG Binding Buffer into each of the individual blue tubes.

3. Aliquot 250µl of diluted sample into each of the individual yellow tubes. Diluted sample volumes exceeding 250µl must be processed using multiple diluted sample fractions.

4. Aliquot 250µl of Protein A IgG Wash Buffer into each of the individual green tubes.

5. Aliquot 200µl of IgG Elution Buffer into each of the individual orange tubes.

6. Carefully remove the upper strip-caps from the Aspire Protein A tips. If less than 8 tips are used, the strip-caps can be carefully separated between adjacent tips with a razor blade prior to removal of the caps.

7. The Aspire tips are sealed at their distal ends with small removable caps to prevent buffer leakage. These caps must be individually removed prior to use.

8. Adjust pipette setting to 300 - 400µl and attach Aspire tip(s) to the pipette.
Note: Determine optimal pipette volume setting by ensuring that the buffer travels entirely through the resin bed without significant bubble formation. This volume setting may vary slightly depending on the pipette model. Once the optimal volumetric setting has been determined, it will remain unchanged throughout the remainder of this procedure.

9. Expel resin storage buffer from the Aspire tip(s) into a waste container.

10. Equilibrate Aspire tip(s) by aspirating the entire volume of Protein A IgG Binding Buffer from the blue tube (row A). Expel solution back into the same tube. Repeat aspirating/dispensing once with the same fraction of Protein A IgG Binding Buffer.
Note: During aspiration, allow the buffer to pass through the entire resin bed before dispensing.

11. Insert Aspire tip(s) into the yellow tube. Bind IgG by aspirating the diluted sample from the yellow tube (row B). Expel sample back into the same tube. Repeat aspirating/dispensing at least 3 more times, for a minimum of 4 times total.

12. Repeat step 11 with any additional sample fractions used.

13. Insert Aspire tip(s) into the green tube (row C). Wash by aspirating 250µl of Protein A IgG Wash Buffer. Expel solution back into the same tube. Repeat once with the same 250µl fraction of Protein A IgG Wash Buffer.

14. Repeat step 13 using the Protein A IgG Wash Buffer in row D.

15. Repeat step 13 using the Protein A IgG Wash Buffer in row E. Make sure the Wash Buffer is completely expelled before proceeding to the next step.

16. Insert Aspire tip(s) into the orange tube (row F). Elute IgG by aspirating 200µl of IgG Elution Buffer. Expel solution back into the same tube. Repeat aspirating/dispensing at least 3 more times, for a minimum of 4 times total.

17. Repeat step 16 using IgG Elution Buffer in row G.
Note: Most of the bound IgG should be eluted in the first two elution fractions.

18. Immediately neutralize samples by adding 20µl of IgG Neutralization Buffer to each elution fraction.

19. Pool elution fractions if desired and transfer into micro-centrifuge tubes for storage. **Note:** For long-term storage, freeze purified IgG samples at -20^{o}C.

20. Discard used colored tubes, Aspire tips and buffers appropriately.

Procedure Using a Thermo Scientific Finnpipette Novus Electronic Pipette

A. Additional Materials Required

- 1000µl single or multichannel electronic Novus pipette

B. Material Preparation

1. **1X Sample Dilution Buffer:** Dilute 200X Sample Dilution Buffer to 1X in Protein A IgG Binding Buffer. Vortex to mix. The total volume needed is determined by the quantity of tips being used and the volume of sample being processed per tip.
   Note: This mixture should be prepared fresh prior to sample prep.

2. **Sample Dilution:** Dilute the sample a minimum of 1:1 in 1X Sample Dilution Buffer (prepared in step 1) to a total volume of 250µl per fraction.
   For example: Add 125µl of sample to 125µl of 1X Sample Dilution Buffer or add 25µl of sample to 225µl of 1X Sample Dilution Buffer.
   Note: Each tip has the capacity to purify 1mg of human IgG. Diluted sample volumes exceeding 250µl must be processed using multiple diluted sample fractions. Additional yellow tubes are provided to prepare two sample fractions per tip.

C. Purification Protocol

Note: Procedure can be performed at room temperature or 4°C.

1. Arrange the colored tubes in the provided rack as illustrated in diagram 1 on page 2. Each sample requires the following quantities of colored tubes for processing:
   - 1 blue
   - 1 yellow
   - 3 green
   - 2 orange

   Note: Up to 8 samples can be processed in parallel using a multichannel pipette.

2. Using the Finnpipette Novus electronic multichannel pipette, aliquot 250µl of Protein A IgG Binding Buffer into each of the individual blue tubes.

3. Aliquot 250µl of diluted sample into each of the yellow individual tubes.

4. Aliquot 250µl of Protein A IgG Wash Buffer into each of the individual green tubes.

5. Aliquot 200µl of IgG Elution Buffer into each of the individual orange tubes.

6. Carefully remove the upper strip-caps from the Aspire Protein A Tips. If less than 8 tips are used, the strip-caps can be separated between adjacent tips with a razor blade prior to removal of the caps.

7. The Aspire tips are sealed at their distal ends with small removable caps to prevent buffer leakage. These caps must be individually removed prior to use.

8. Using the ***STEPPER*** function on the Novus pipette, adjust pipette setting to 1x 300 – 1x 400µl and attach Aspire tip(s) to the pipette. Adjust the aspirate and dispense rates to 9 on the Novus pipette as illustrated on Figure 1.
Note: The ***STEPPER*** function is used as it allows for a *blowout* step which ensures complete dispensing of all aspirated liquid.

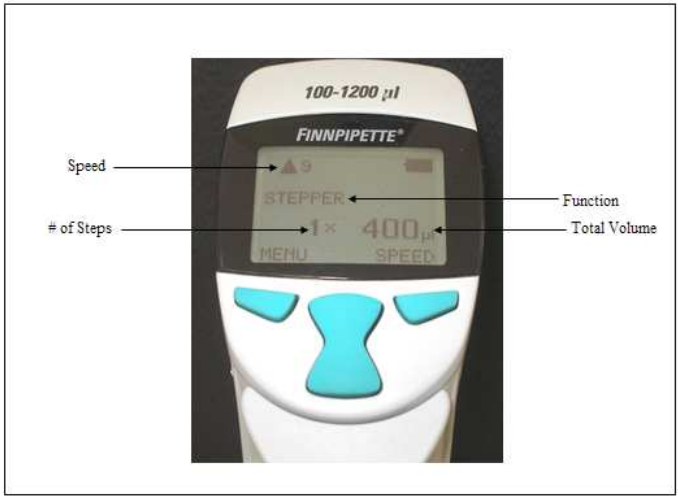


Figure 1. Finnpipette Novus electronic multichannel showing the ***STEPPER*** function

9. Prior to attaching tip(s) to the Novus pipette, depress the trigger button once to aspirate, then ensure that the pipette is set to dispense. This will facilitate expelling the storage buffer contained in the tips.

10. Firmly attach the Aspire Protein A tip(s) to the Novus pipette. Depress the trigger button to expel the storage buffer into a waste container.

11. Equilibrate Aspire tip(s) by aspirating the entire volume of Protein A IgG Binding Buffer from the blue tube (row A). Expel solution back into the same tube. Repeat aspirating/dispensing once with the same fraction of Protein A IgG Binding Buffer.
Note: For the most effective dispense, depress the trigger button once and allow the buffer level to reach the top edge of the upper filter, release trigger, depress again and hold the trigger button until the remainder of buffer is completely expelled.

12. Insert Aspire tip(s) into the yellow tube. Bind IgG by aspirating the diluted sample from the yellow tube (row B). Expel sample back into the same tube. Repeat aspirating/dispensing at least 3 more times, for a minimum of 4 times total.

13. Repeat step 12 with any additional sample fractions used.

14. Insert Aspire tip(s) into the green tube (row C). Wash by aspirating 250µl of Protein A IgG Wash Buffer. Expel solution back into the same tube. Repeat once with the same 250µl fraction of Protein A IgG Wash Buffer.

15. Repeat step 14 using the Protein A IgG Wash Buffer in row D.

16. Repeat step 14 using the Protein A IgG Wash Buffer in row E. Make sure the Wash Buffer is completely expelled before proceeding to the next step.

17. Insert Aspire tip(s) into the orange tube (row F). Elute IgG by aspirating 200µl of IgG Elution Buffer. Expel solution back into the same tube. Repeat aspirating/dispensing at least 3 more times, for a minimum of 4 times total.

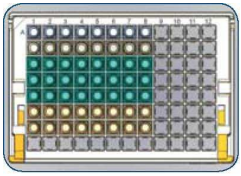
18. Repeat step 17 using IgG Elution Buffer in row G.
Note: Most of the bound IgG should be eluted in the first two elution fractions.

19. Immediately neutralize samples by adding 20µl of IgG Neutralization Buffer to each elution fraction.

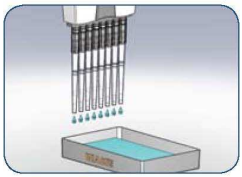
20. Pool elution fractions if desired and transfer into micro-centrifuge tubes for storage.
Note: For long-term storage, freeze purified IgG samples at -20^{o}C.

21. Discard used colored tubes, Aspire tips and buffers appropriately.

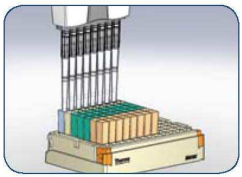
D. Procedure Flowchart



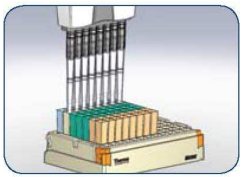
Prepare Tube Rack
Arrange the colored tubes in the provided rack. Fill tubes with the appropriate volumes of solutions.



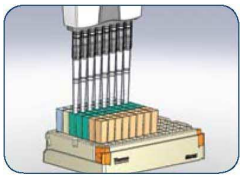
Expel Storage Buffer
Attach pipette to Aspire tips and dispense storage buffer into waste container. **Novus Electronic Pipette:** Use STEPPER mode and adjust the setting to 1x 300 - 1x 400 µl. Aspirate prior to attaching Aspire tips, attach tips then dispense storage buffer into waste container.



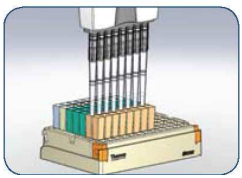
Equilibrate (Row A, blue tube)
Equilibrate the resin by aspirating and dispensing Protein A Binding Buffer 2X.



Bind IgG (Row B, yellow tube)
Aspirate and dispense each diluted sample fraction a minimum of 4X.



Wash (Rows C through E, green tubes)
Aspirate and dispense each wash fraction 2X.



Elute IgG (Rows F and G, orange tubes)
Aspirate and dispense each elution fraction a minimum of 4X.

Neutralize
Immediately neutralize elution fractions by adding the appropriate volume of neutralization buffer

Troubleshooting

Problem	Potential Cause	Solution
Considerable antibody purified, but no specific antibody of interest detected	Antibody of interest is at very low concentration	Use serum-free medium for cell supernatant samples
		Use specific antigen to selectively purify the antibody
Poor or incomplete sample recovery	pH of IgG Wash Buffer is too low	Check wash fractions for presence of IgG. Wash fraction pH should be ≈ 7.9-8.1
	pH of IgG Elution Buffer is too high	Elute sample in fresh aliquot of IgG Elution Buffer. Ensure that pH ≈ 2.5-3.0
Bubbles created inside the tips	Binding Buffer not prepared properly	Ensure the binding solution is being prepared correctly by mixing the Sample Dilution Buffer and Binding Buffer together in the proper ratio
	Pipette volume set too high	Reduce the volume setting on pipette to a level where the solutions are passing through the resin bed without creating bubbles

Related Thermo Scientific Products

990-02 **Thermo Scientific Aspire Protein G Tips**

4630080X* **Thermo Scientific Finnpipette® Novus Multichannel Electronic Pipette**
(8-channel, 100-1200µl)

*** X: 0=Eur, 0=U.S., 2=Japan, 3=U.K. and 4=Australia**

MSDS Information

The Material Safety Data Sheet (MSDS) information for the products are included with the product shipment and also available electronically at www.thermo.com. Simply navigate to the product page to retrieve any associated MSDS's in a print format.

Expiration Period

Six month from date of sale for product used, handled and stored according to manufacturer instructions, see details under **Warranty**.

The most current versions of all Aspire product instructions are available at www.thermo.com. For a copy, please go to www.thermo.com or your local distributor.

Warranty: Sellers warrants that the Products will operate or perform substantially in conformance with Seller's published specifications and be free from defects in material and workmanship, when subjected to normal, proper and intended usage by properly trained personnel, for the period of time set forth in the product documentation, published specifications or page insert. If a period of time is not specified in Seller's product documentation, published specifications or package inserts, the warranty period shall be one (1) year from the date of shipment to Buyer for equipment and ninety (90) days for all other products (the "Warranty Period"). Seller agrees the Warranty Period, to repair or replace at Seller's option, defective Products so as to cause the same to operate in substantial conformance with said published specifications, provided that Buyer shall (a) promptly notify Seller in writing upon the discovery of any defect, which notice shall include the product model and serial number (if applicable) and details of the warranty claim, and (b) after Seller's review. Seller will provide Buyer with service data and/or a Return Material Authorization ("RMA"), which may include biohazard decontamination procedures and other product-specific handling instructions, then, if applicable, Buyer may return the effective Products to Seller with all costs prepaid by Buyer. Replacement parts may be new or refurbished at the election of Seller. All replaced parts shall become the property of Seller. Shipment to Buyer repaired or replacement Products shall be made in accordance with the Delivery provisions of the Seller's Term and Conditions of Sale. Consumables are expressly excluded this warranty.
Notwithstanding the foregoing, Products supplied by Seller that are obtained by Seller from an original manufacturer or third party supplier are not warranted by Seller, but Seller agrees to assign to Buyer any warranty rights in such Product that Seller may have from the original manufacturer or third party supplier, to the extent such assignment is allowed by such original manufacturer or third party supplier.

In no event shall Seller have any obligation to make repairs, replacements or corrections required, in whole or in part, as the result as (i) normal wear and tear, (ii)accident, disaster or event of force majeure, (iii)misuse, fault or negligence of or by Buyer, (iv)use of the Products in a manner for which they were not designed, (v) causes external to the Products such as, but not limited to, power failure or electrical power surges, (vi)improper storage and handling of the Products or (vii) use of the Products in combination with equipment or software not supplied by Seller. If Seller determines that Products for which Buyer has requested warranty services are not covered by the warranty hereunder. Buyer shall pay or reimburse Seller for all costs of investigating and responding to such request at Seller's then prevailing time and materials rates. If Seller provides repair services or replacement parts that are not covered by this warranty, Buyer shall pay Seller therefore at Seller's then prevailing time and materials rates. Any installation, maintenance, repair, service, relocations or alteration to or of, or other tampering with, the products preformed by any person or entity other than seller without sellers's prior written approval, or any use of replacement parts not supplied by seller, shall immediately void and cancel all warranties with respect to the affected products. The obligations created by this warranty statement to repair or replace a defective product shall be the sole remedy of buyer in the event of defective product except as expressly provided in this warranty statement, seller disclaims all other warranties, whether express or implied, oral or written, with respect to the products, including without limitations all implied warranties of merchantability or fitness for any particular purpose. Seller does not warrant that the products are error-free or will accomplish any particular result.

The most current versions of all Aspire product instructions are available at www.thermo.com. For a copy, please go to www.thermo.com or your local distributor.

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