User Guide

Amicon® Pro Purification System

A Centrifugal Tool for the Sample Prep Workflow

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Contents

Introduction

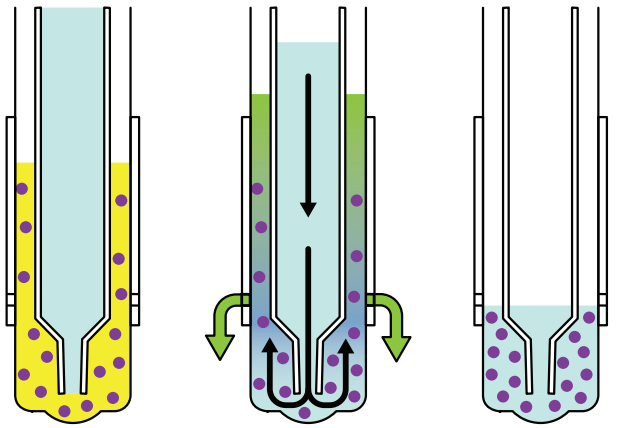
Affinity purification is based on the specific interaction of a target molecule with an immobilized ligand. For recombinant proteins, the addition of fusion tags enables affinity purification by a number of strategies, including resin-based matrices. Current small-scale centrifugal-based strategies are labor intensive, requiring multiple spins at each step and numerous sample transfers between different devices. This is particularly true in cases where samples must be dialyzed (buffer exchange) and/or concentrated prior to downstream application. The Amicon® Pro Purification System streamlines this process by providing a single adaptable centrifugal device capable of performing all steps in the purification scheme. When used in combination with affinity resins, this system offers a robust, time-effective solution for small-scale purification of affinity-tagged proteins.

By condensing the entire purification workflow into one device, the Amicon® Pro Purification System eliminates the need for multiple sample transfers, thereby minimizing protein loss. The large exchange reservoir accommodates a range of volumes (up to 10 mL) as well as reducing the need for multiple spin steps during the wash and elution phases. Direct coupling to an Amicon® Ultra-0.5 device further provides simultaneous concentration during the elution phase; a feature particularly advantageous for depletion or enrichment applications. Lastly, the Amicon® Pro Purification System offers highly efficient diafiltration, permitting buffer exchange and concentration of the final purified sample in a single 15 minute spin.

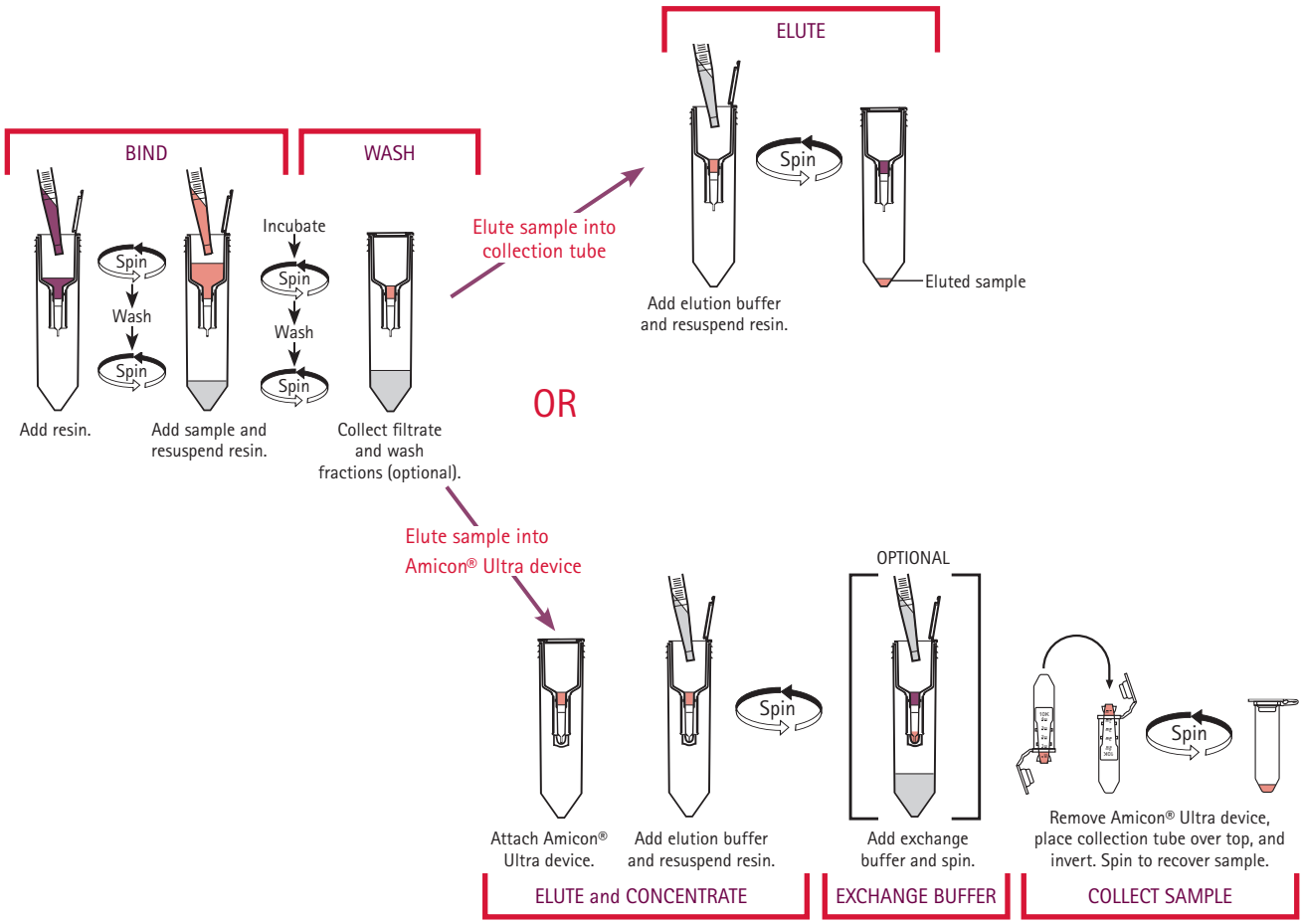
Design Features

The Amicon® Pro system builds on key attributes of the Amicon® Ultra-0.5 device; fast, efficient sample concentration with minimal membrane fouling or sample loss. The lower portion of exchange device is designed to exactly match the contours of Amicon® Ultra-0.5 mL filter. This adaptation facilitates optimal exposure of the filtering membrane's active surface area while reducing space (volume) between the plastic sidewalls. The tip is also tapered to maximize the external-to-internal volume ratio, ensuring that fresh buffer is slowly but consistently metered in, mixed with sample, then forced across the membrane boundaries. This continuous process decreases the likelihood of protein aggregation or precipitation. Moreover, the upper reservoir of the Amicon® Pro device is designed to hold up to 10 mL of buffer, permitting single-spin exchange and concentration.

Concentration and exchange in a single spin



The Amicon® Pro Purification System Workflow



Applications

Due to its modular design, the Amicon® Pro device can be used for both affinity-based purification applications and filter-based applications that do not require up-front purification.

Affinity-based applications

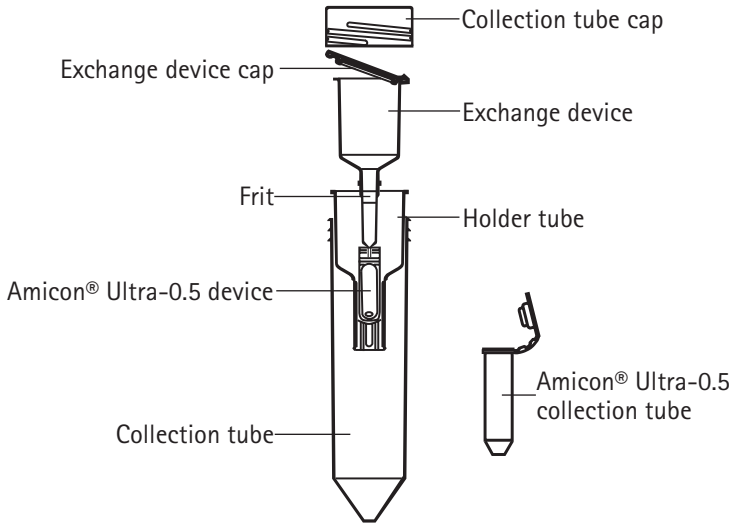
- Affinity-based protein purification with Amicon® Pro Purification Kits: Each kit contains resin and buffers and can be ordered with and without the Amicon® Pro Purification System. Refer to Ordering Information section for available kits.
- Affinity-based purification using other commercially available resins (greater than 10 µm in diameter) and buffers
- Affinity-based depletion of biological samples (serum) for downstream analysis

Filter-based applications without purification (requires Amicon® Ultra-0.5 device)

- Concentration of biological samples containing proteins, nucleic acids (DNA/RNA samples, either single- or double-stranded), and microorganisms
- Small-scale antibody conjugation
- Desalting, buffer exchange, or diafiltration for sample cleanup

Parts and Functions of the Amicon® Pro Purification System

The Amicon® Pro Purification System consists of the following components:



Part	Function
Collection tube cap	Collection tube sample containment
Exchange device cap	Exchange device sample containment, also acts as a diaphragm to displace residual sample from the tip of the exchange device
Exchange device	Facilitates capture, wash, and elution of protein sample
Frit	Low protein-binding, moderately hydrophobic disc holds back aqueous solutions until centrifugation and prevents resin passage during centrifugation
Holder tube	Seats exchange device in 50 mL collection tube and provides attachment for Amicon® Ultra device
Amicon® Ultra-0.5 device	Used to concentrate the sample with the option of simultaneous buffer exchange. The device is capable of 30-fold concentration, with high sample recovery (typically greater than 90% of dilute starting solution). Refer to the Amicon® Ultra-0.5 User Guide for further information. The Amicon® Ultra-0.5 device is available in 5 different cutoffs (molecular weight cutoff, MWCO). 3K device – 3,000 MWCO 10K device – 10,000 MWCO 30K device – 30,000 MWCO 50K device – 50,000 MWCO 100K device – 100,000 MWCO
Amicon® Ultra-0.5 collection tube	Used for final sample recovery by reverse spin from the Amicon® Ultra-0.5 device
Collection tube	Collects sample flow-through, wash fractions, and Amicon® Ultra-0.5 ultrafiltrate during the various steps of the protocol

Materials Required but Not Supplied

- Centrifuge with swinging-bucket rotor that can accommodate 50 mL conical tubes, and is rated for at least 4,000 × g
- Microcentrifuge with fixed-angle rotor that can accommodate 1.5 mL microcentrifuge tubes (for Amicon® Ultra-0.5 device reverse spin), rated for 14,000 × g

  CAUTION: To avoid damage to device components during centrifugation, check clearance before spinning.

- Plate shaker with speed control

Device Storage

Store all device components at room temperature.

NOTE: The frit in the neck of the exchange device may yellow slightly with age, but this does not impact device integrity or performance in any of the defined applications.

Device Sanitization

The Amicon® Pro Purification System can be sanitized with 70% ethanol, however, the ethanol only sanitizes the device; it does not sterilize it. The device is **not** autoclavable.

How to use the Amicon® Pro Purification System

The Amicon® Pro Purification System is compatible with most commercially available resins greater than 10 µm in diameter, and buffers commonly employed in protein purification systems. The device is also available as part of an optimized kit that includes resins and buffers (refer to Ordering Information). To ensure optimal performance, please consult the chemical compatibility table prior to use.

Assembly

1. Insert exchange device into the holder tube.
2. Place the exchange device/holder tube assembly in the collection tube.
3. Set Amicon® Ultra-0.5 device aside until needed for the concentration/buffer exchange step.

   NOTE: The ultrafiltration membranes in the Amicon® Ultra-0.5 devices contain trace amounts of glycerine. If this material interferes with analysis, rinse the device with buffer or Milli-Q® water. If interference continues, rinse with 0.1 N NaOH followed by a second spin of buffer or Milli-Q® water. Do not allow the membrane in Amicon® Ultra filter devices to dry out once wet. If you are not using the device immediately after rinsing, leave fluid on the membrane until the device is used.

Affinity Purification

The protocol outlined below is based on the purification of a tagged-recombinant protein from 0.5 mL *E. coli* lysate using 100 µL resin (200 µL of 50% slurry). Due to large variability in protein samples, no single protocol can provide optimal results in all cases. Parameters which may require optimization include cell growth/induction, sample preparation method, resin input, binding reaction time, and wash and elution steps. In addition to standard elution, the protocol also includes steps for simultaneous sample concentration during the elution step, using the Amicon® Ultra-0.5 device.

NOTE: The Amicon® Pro Purification System is designed specifically for use in a centrifuge with a swinging-bucket rotor. Centrifugation in a fixed-angle format results in reduced protein recovery due to failure of the resin to pack tightly at the base of the exchange device. Buffer exchange and concentration capacity are also impacted if devices are spun in a fixed-angle rotor.

Resin Preparation

1. To ensure uniform suspension, vortex the resin thoroughly before adding it to the device.
2. Remove collection tube cap and open the exchange device cap.
3. Add 100 µL of resin (200 µL of 50% slurry) to the base of the exchange device. Close the exchange device cap.

   NOTE: Up to 1,000 µL of packed resin may be added to the device.

   Wide-bore tips are recommended for resin transfer.
4. To remove the storage buffer, centrifuge in a swinging-bucket rotor at 1,000 × g for 1 minute.

   NOTE: Given the large volume of the collection tube (50 mL), it is not necessary to discard the filtrate following each centrifugation step. However, if you wish to save each individual fraction (e.g., flow-through and wash fractions), empty the collection tube between steps.
5. Add 500 µL (equivalent to 5 packed volumes resin) of the appropriate equilibrium/binding buffer. Centrifuge at 1,000 × g for 1 minute.

Protein Binding and Wash

1. Add 500 µL of sample to base of the exchange device and mix with packed resin by pipetting.

   NOTE: Approximately 9 mL of sample can be added; the volume loaded is determined by the target protein's expression level and/or resin binding capacity.
2. Incubate for 60 minutes at room temperature with gentle agitation (standard plate shaker at low setting).

   NOTE: Duration of binding time will vary with the application.

   For larger volumes and extended binding reactions, mixing by end-over-end inversion may be preferred. In such cases, we recommend sealing the exchange device cap with tape over the vent hole (remove tape prior to centrifugation). We do not recommend end-over-end mixing with small volumes; due to insufficient volume, substantial amounts of resin may be lost to the sides of the exchange device.

Affinity Purification, continued

3. Centrifuge device at 1,000 × g for 1 minute in a swinging-bucket rotor. Recover sample flow-through if desired.

   NOTE: For reactions using ≥ 500 µL of packed resin, we recommend centrifuging at 2,000 × g for 4 minutes to ensure filtrate clearance.

   To maximize protein capture, collect all resin into solution prior to centrifugation.

4. Add 1.5 mL bind/wash buffer and centrifuge at 1,000 × g for 1 minute (2,000 × g for 4 minutes if using ≥ 500 µL of packed resin). Recover wash fraction if desired.

   NOTE: Due to the large capacity of the exchange device, the volume of the wash can be increased for greater sample purity. There is no need for multiple wash steps.

Sample Elution

Samples can be eluted without concentration by adding elution buffer and centrifuging at 1,000 × g for 2 minutes (2,000 × g for 4 minutes if using ≥ 500 µL of packed resin) directly into a clean 50 mL collection tube. If the expected protein yield is greater than 1 mg, we recommend eluting directly into a clean collection tube rather than into the Amicon® Ultra-0.5 device.

NOTE: For more information on using the Amicon® Ultra-0.5 device, please refer to the Amicon® Ultra-0.5 User Guide, available at www.millipore.com. Enter *Amicon Ultra 0.5 User Guide* in the search box.

1. Remove the exchange device from the holder tube and insert it into the Amicon® Ultra-0.5 device.

   NOTE: To avoid damaging the membrane surfaces within the Amicon® Ultra-0.5 device, use caution during insertion of the exchange device tip.

2. Place the exchange device/Amicon® Ultra-0.5 assembly back into the holder tube and return this assembly to the collection tube.

3. Add up to 1.5 mL of elution buffer and gently resuspend the resin.

   NOTE: Under standard conditions, one elution is sufficient for recovery of 90–95% of captured protein. The elution volume can be increased if required.

4. Recap the collection tube and centrifuge the device at 4,000 × g for 15 minutes in a swinging-bucket rotor.

   NOTE: Depending on the starting elution volume, the MWCO of the Amicon® Ultra-0.5 device employed, and the degree of concentration desired, the length of the spin time can range from 10 to 30 minutes. Refer to the Performance Characteristics section for recommended guidelines.

Affinity Purification, continued

Desalting, buffer exchange, or diafiltration are important methods for removing salts or solvents from solutions containing biomolecules. The removal of salts or exchange of buffers can be accomplished in the Amicon® Pro Purification System by first concentrating the sample (as outlined above) then reconstituting the sample with any desired solvent. Due to the expanded size of the exchange device, the buffer exchange process can be accomplished with a single centrifugation.

Buffer Exchange and Concentration (optional)

1. After simultaneous sample elution with concentration, add 1.5 mL of the desired buffer to the exchange device/Amicon® Ultra-0.5 assembly.
2. Centrifuge device at 4,000 × g for 15 minutes in a swinging-bucket rotor. Concentrated samples can be recovered from the Amicon® Ultra-0.5 device by reverse spin (see below).

Collecting Sample from Amicon® Ultra-0.5 Device by Reverse Spin

1. Remove the exchange device/Amicon® Ultra-0.5 assembly from the holder tube and disassemble.
2. Using a gentle twisting motion, detach the Amicon® Ultra-0.5 device from the exchange device.
3. If there is residual sample in the exchange device tip, depress the exchange device cap to expel the remaining sample volume into the Amicon® Ultra-0.5 device.
4. Hold the Amicon® Ultra-0.5 device upright and place the 2 mL collection tube over the top.
5. Invert the assembly and centrifuge in a microcentrifuge with fixed-angle rotor 1,000 × g for 2 minutes.

Sample protein yield can be determined by infrared (IR)-based spectrometry using the Direct Detect® System and Assay-free cards.

Buffer Exchange

Due to its modular design, the Amicon® Pro device can also be used for filter-based applications that do not require up-front purification. Building on key attributes of the Amicon® Ultra-0.5 filter, namely fast, efficient sample concentration with minimal membrane fouling or sample loss, the Amicon® Pro device brings a larger upper exchange reservoir (up to 10 mL) for gentle sample processing via continuous diafiltration in a single spin. Potential applications includes: (1) desalting/buffer exchange, (2) small-scale antibody conjugation, and (3) concentration of biological samples containing antibodies, enzymes, nucleic acids, microorganisms, and purified proteins. Protocols for buffer exchange and concentration for dilute (or large volume) and concentrated samples are detailed below.

For dilute or large volume samples

1. Attach the Amicon® Ultra-0.5 filter to the base of exchange device.
2. Add 0.5 mL TBST [1% Tween® 20 surfactant in tris-buffered saline (vol/vol)] to the exchange device. Centrifuge at 4,000 × g for 1 minute.
3. **Optional:** Add 0.5 mL of tris-buffered saline (TBS) or appropriate buffer and centrifuge at 4000 × g for 1 minute.

Buffer Exchange, continued

4. Clear any residual buffer from the Amicon® Ultra-0.5 filter by reverse spin (see above).

   NOTE: Prewetting (steps 2–4) is performed to minimize potential non-specific binding to the frit. If concerns exist over detergent interference in downstream applications, an optional wash (step 3) can be included to remove residual Tween® 20 surfactant.

5. Add sample to the exchange device and centrifuge at 4,000 × g. Refer to Figure 1 in the Flow Rate section for spin times to achieve desired final concentrate volume.
6. After sample concentration, add 1.5 mL of the desired buffer to the exchange device/Amicon® Ultra-0.5 assembly.
7. Centrifuge device at 4,000 × g for 15 minutes in a swinging-bucket rotor. Concentrated samples can be recovered from the Amicon® Ultra-0.5 device by reverse spin (see above).

For concentrated samples (≤ 200 µL)

1. Load sample into the Amicon® Ultra 0.5 filter and attach it to the base of the exchange device.
2. Add 1.5 mL of the desired buffer to the exchange device/Amicon® Ultra-0.5 assembly.
3. Centrifuge device at 4,000 × g for 15 minutes in a swinging-bucket rotor. Concentrated samples can be recovered from the Amicon® Ultra-0.5 device by reverse spin (see above).

Sample protein yield can be determined by infrared (IR)-based spectrometry using the Direct Detect® System and Assay-free cards.

Sample Depletion or Enrichment

Human serum and plasma are rich sources of proteomic information often interrogated for biomarkers of physiological and disease states. While easily obtainable in sufficient quantities, one of the major challenges in analyzing such complex samples is the wide concentration range of proteins present. Abundantly expressed species, such as albumin or immunoglobulins, can constitute up to 70% of total protein in these fluids. In contrast, protein markers of clinical interest may be present at significantly lower concentrations (pg/mL – ng/mL range). Depletion of these highly abundant components is required for identification and accurate quantitation of rarer protein species. Outlined below is a general protocol for immunoglobulin depletion from serum using 100 µL of packed resin. Due to large variability between samples, no single protocol can provide optimal results in all cases. Parameters that may require optimization include the ratio of sample to resin, binding reaction time, and degree of washing.

Resin Preparation

Refer to Resin Preparation in Affinity Purification section.

Sample Depletion or Enrichment, continued

Protein Binding and Sample Depletion with Concentration

Depleted fractions can be collected without concentration by centrifuging at 1,000 × g for 2 minutes directly into a clean 50 mL collection tube. For reactions using ≥ 500 µL of packed resin, we recommend centrifuging at 2,000 × g for 4 minutes to ensure filtrate clearance.

1. For simultaneous sample depletion with concentration, attach the Amicon® Ultra-0.5 device to the exchange device, place this assembly back into the holder tube, and then into the 50 mL collection tube, as described previously.

   NOTE: For starting sample volumes greater than 1.5 mL, we recommend sample depletion without simultaneous concentration. Do not attach the Amicon® Ultra-0.5 device, but instead, deplete the sample directly into the collection tube.

2. Add resin slurry to the exchange device, remove storage buffer, and wash as previously described.

   NOTE: Depending on the type of sample, depletion target level, and resin binding capacity, the amount of resin required can vary greatly.

   If the Amicon® Ultra 0.5 device is not being used, aspirate buffer from the collection tube.

3. Add 500 µL of sample to the base of the exchange device and mix with packed resin by pipetting.

   NOTE: Depending on depletion target concentration, the starting sample may need to be diluted prior to loading.

   Approximately 9 mL of sample can be added; the volume loaded is determined by the target protein's expression level and/or resin binding capacity.

4. Incubate for 60 minutes at room temperature with gentle agitation (standard plate shaker at low setting).

   NOTE: Duration of binding time will vary with the application.

   For larger volumes and extended binding reactions, mixing by end-over-end inversion may be preferred. In such cases, we recommend sealing the exchange device cap with tape over the vent hole (remove tape prior to centrifugation). We do not recommend end-over-end mixing with small volumes; due to insufficient volume, substantial amounts of resin may be lost to the sides of the exchange device.

5. Centrifuge device at 4,000 × g for 15 minutes in a swinging-bucket rotor. A wash step may now be performed (see below) or concentrated samples can be buffer-exchanged and recovered from the Amicon® Ultra-0.5 device by reverse spin as described previously.

   NOTE: If the Amicon® Ultra-0.5 device is not being used, centrifuge at 1000 × g for 2 minutes. The depleted sample can be removed from the collection tube by pipetting.

Sample Depletion or Enrichment, continued

Wash Steps for Residual Sample Recovery

To maximize recovery of proteins in the depleted fraction, under certain conditions, it may be advantageous to wash the resin prior to final sample recovery. Washing should be performed immediately following the depletion step. For reactions using ≥ 500 µL of packed resin where the Amicon® Ultra-0.5 device has not been attached, we recommend centrifuging at 2,000 × g for 4 minutes to ensure filtrate clearance.

1. Add 1.5 mL of the appropriate wash buffer to the exchange device.
2. Centrifuge the device at 4,000 × g for 15 minutes in a swinging-bucket rotor. Concentrated samples can be buffer exchanged or recovered from the Amicon® Ultra-0.5 device by reverse spin.

   NOTE: Depending on the starting elution volume, the MWCO of the Amicon® Ultra-0.5 device employed, and the degree of concentration desired, the length of the spin time can range from 10 to 30 minutes. Refer to the Performance Characteristics section for recommended guidelines.

Antibody Labeling

Biological research often requires the use of labeled antibodies or proteins to facilitate detection, quantitation, and/or localization of specific target proteins. Labeling strategies result in the covalent attachment of different molecules, including biotin and fluorophores, to the target protein. The standard labeling protocol routinely involves initial sample preparation, labeling, removal of excess label, dialysis, and final concentration. In most cases, this process is lengthy, tedious, and subject to significant protein loss due to multiple transfers between devices. The Amicon® Pro Purification System enables small-batch labeling of antibodies in approximately 1 hour. Outlined below is a general protocol for biotinylation of 50 µg of antibody using the Innolink™ Biotin 354S kit (EMD Millipore Corporation cat. no. 203119). Due to large variability between conjugate properties, kit parameters, and protein targets, no single protocol can provide optimal results in all cases. Parameters which may require optimization include the ratio of antibody to label, binding reaction time, and degree of washing.

Preparation (Buffer Exchange) and Labeling

1. Remove the exchange device from the holder and insert it into the Amicon® Ultra-0.5 device.

   NOTE: For antibody labeling, we recommend using the Amicon® Ultra-0.5 10K device.

   If the antibody does not require initial concentration (≥ 1 mg/mL), the sample (50 µL) can be loaded directly into the Amicon® Ultra-0.5 device prior to attachment to the exchange device. In this case, the device does not require prewetting. Proceed to Step 4.
2. Place exchange device/Amicon® Ultra-0.5 assembly back into the holder tube and return this assembly device to the collection tube.
3. Prewet the device using TBST as previously described in the Buffer Exchange section.

Antibody Labeling, continued

4. Add 50 µg of antibody to the exchange device and centrifuge at 4,000 × g for 15 minutes in a swinging-bucket rotor.
5. Prepare a reaction cocktail containing 1.5 mL of the desired reaction buffer (e.g., 100 mM phosphate-buffered saline (PBS), pH 8) and label (0.2 mg/mL Innolink™ Biotin 354S kit) per labeling reaction.
6. Add 1.5 mL of reaction cocktail to each exchange device. Close the exchange cap and screw on the collection tube cap.
7. Centrifuge device at 4,000 × g for 15 minutes in a swinging-bucket rotor.
8. Incubate the labeling reaction in the Amicon® Pro Purification System for an additional 30 minutes.

Removal of Excess Label, Buffer Exchange, and Concentration

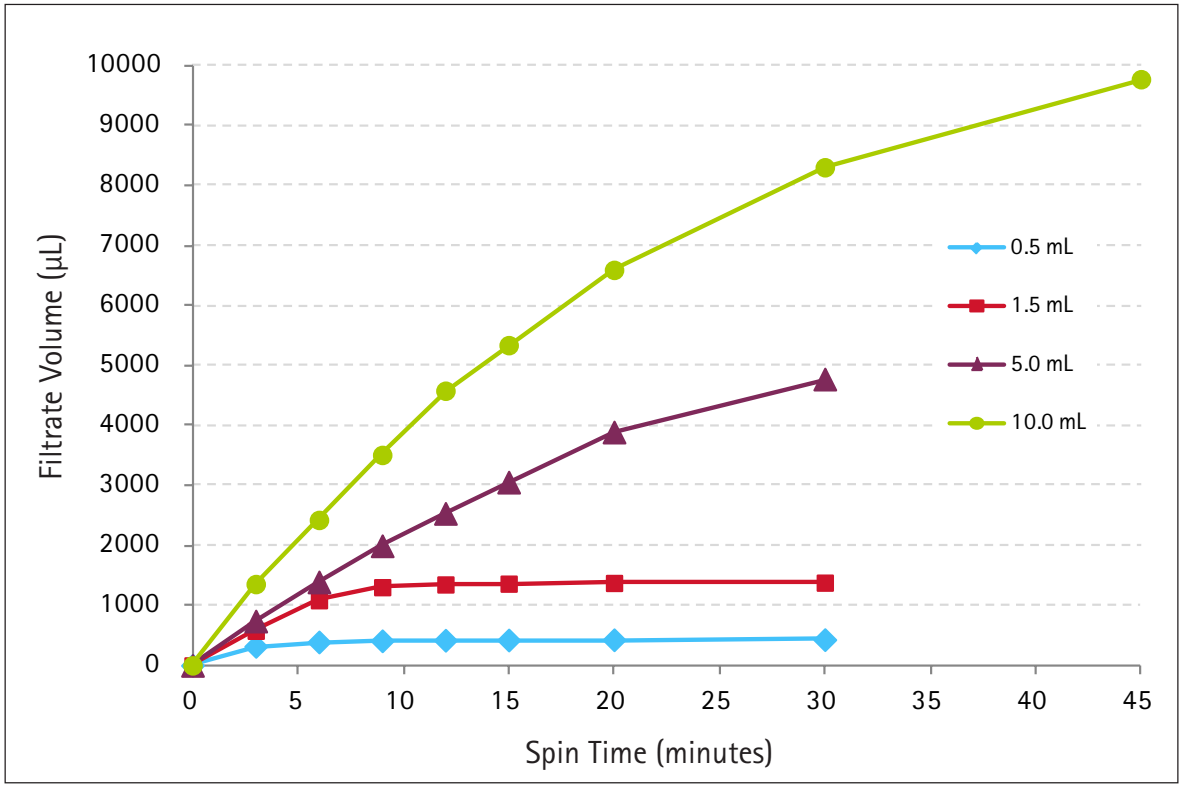
1. Add 1.5 mL of PBS (with or without sodium azide) to the exchange device. Close the exchange device cap and screw on the collection tube cap.
2. Centrifuge device at 4,000 × g for 15 minutes in a swinging-bucket rotor.
3. Recover the labeled antibody from the Amicon® Ultra-0.5 device by reverse spin.
4. The labeled antibody is now ready for downstream applications.

Performance Characteristics

Flow Rate

Factors affecting flow rate include sample concentration, starting volume, chemical nature of solute, relative centrifugal force, membrane type, and temperature. The figures and tables below can be used to estimate the time required to achieve a given volume of filtrate or concentrate. A typical spin time for a 500 µL (0.5 mL) sample is approximately 10 to 30 minutes (depending on device nominal molecular weight limit). While most of the sample is filtered in the first 5 to 10 minutes of centrifugation, the lowest concentrate volume (15–20 µL) is reached after spinning for 30 minutes.

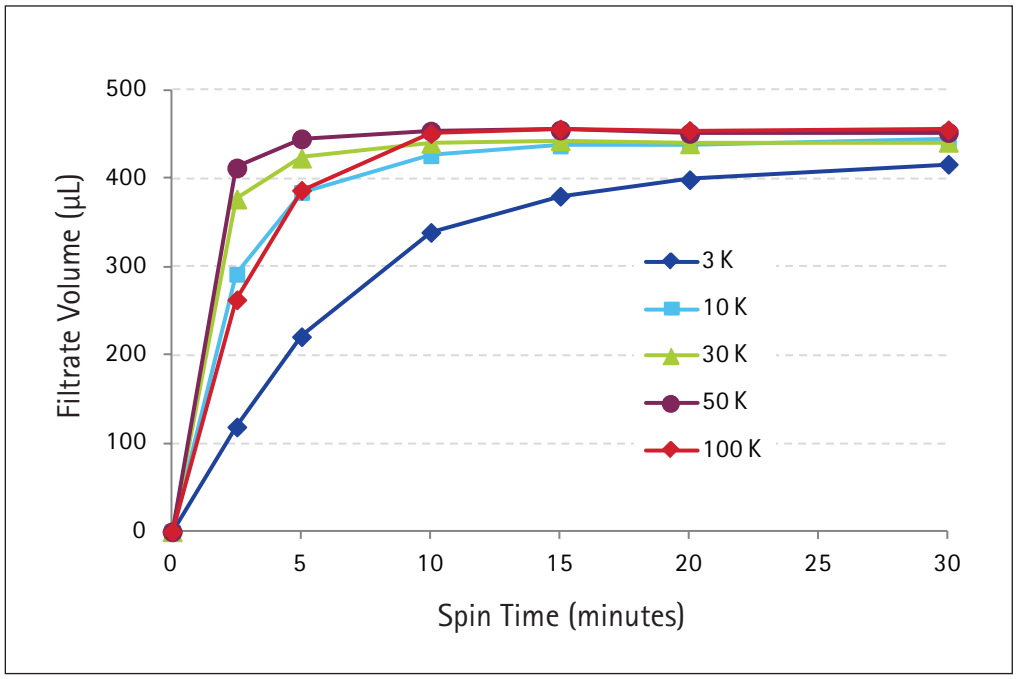
Figure 1. Typical filtrate volume vs. spin time with 0.5, 1.5, 5, and 10 mL starting volumes loaded into the Amicon® Pro Purification System (exchange device coupled to an Amicon® Ultra-0.5 10K device)



Spin conditions: Swinging-bucket rotor, 4,000 × g, room temperature
Protein marker: Bovine serum albumin (BSA), n=4.

Flow Rate, continued

Figure 2. Typical filtrate volume vs. spin time for the Amicon® Pro Purification System (exchange device coupled to an Amicon® Ultra-0.5 3K, 10K, 30K, 50K, or 100K device)



Spin conditions: Swinging-bucket rotor, 4,000 × g, room temperature, 500 µL starting volume. Protein markers used: Lysozyme for 3K and 10K, BSA for 30K and 50K, and IgG for 100K, n=4.

Table 1. Typical Concentrate Volume / Concentration Factor vs. Spin Time

	3K device		10K device		30K device		50K device		100K device	
Spin Time (min)	Conc. Volume (µL)	Conc. Factor (x)	Conc. Volume (µL)	Conc. Factor (x)	Conc. Volume (µL)	Conc. Factor (x)	Conc. Volume (µL)	Conc. Factor (x)	Conc. Volume (µL)	Conc. Factor (x)
5	279	2	115	4	77	7	55	9	113	4
10	161	3	74	7	60	8	46	11	49	10
15	120	4	63	8	57	9	45	11	44	11
20	101	5	61	8	60	8	49	10	45	11
30	84	6	56	9	59	9	48	11	44	11

Spin conditions: Swinging-bucket rotor, 4,000 × g, room temperature, 500 µL starting volume. Protein markers used: Lysozyme for 3K and 10K, BSA for 30K and 50K, and IgG for 100K, n=4.

Protein Retention and Concentrate Recovery

The membranes used in the Amicon® Ultra-0.5 component of the Amicon® Pro Purification System are characterized by a molecular weight cutoff (MWCO); that is, their ability to retain molecules above a specified molecular weight. Solutes with molecular weights close to the MWCO may be only partially retained. Membrane retention depends on the solute's molecular size and shape. For most applications, molecular weight is a convenient parameter to use in assessing retention characteristics. For example, use a membrane with a MWCO at least two times smaller than the molecular weight of the protein solute that one intends to concentrate.

Factors that determine sample recovery include the nature of the protein solute relative to the device MWCO chosen, starting concentration, and concentration factor. Table 2 provides typical recoveries for Amicon® Pro Purification Systems.

Table 2. Typical Concentrate Recovery

Marker/ Concentration	Molecular Weight	Device MWCO	Spin Time (min)	Concentrate Volume (µL)	Concentration Factor (x)	Concentrate Recovery (%)
Lysozyme (1 mg/mL)	14,300	3K	30	84	6	96
Lysozyme (1 mg/mL)	14,300	10K	30	56	9	92
BSA (1 mg/mL)	67,000	30K	30	59	9	90
BSA (1 mg/mL)	67,000	50K	30	48	11	85
IgG (1 mg/mL)	156,000	100K	30	44	11	83

Spin Conditions: Swinging-bucket rotor, 4,000 × g, room temperature, 500 µL starting volume, n=4.

Troubleshooting

Issue: Protein recovery in eluted fraction (or depleted fraction) is low	
Possible Cause	**Solution**
Protein expression was insufficient.	Optimize growth/induction conditions.
Protein was insoluble (inclusion bodies).	Following lysate clearance, check the pellet and supernatant for protein. Perform cell lysis under denaturing conditions.
Protein formed aggregates.	Add solubilizing agents such as detergents, or increase salt concentration of lysis and binding buffers.
Cell lysate was too viscous.	Dilute lysate in binding buffer. Include Benzonase® nuclease in lysis buffer to remove free RNA/DNA.
Protein did not bind to or bound weakly to the resin.	Check the flow-through and wash filtrates for presence of the target protein. Increase the length of binding and optimize buffer conditions for the binding and wash steps.
Insufficient volume of resin was used.	Ensure that the resin slurry is well mixed prior to pipetting. Increase resin volume.
Resin had low binding capacity.	Check the flow-through for presence of the target protein. Increase resin volume.
Insufficient binding reaction time.	Perform the binding reaction for longer than 60 minutes.
Inappropriate elution buffer was used.	Optimize buffer conditions for the elution step.
A fixed-angle rotor was used.	Repeat process using a swinging-bucket rotor.
Sample leaked during end-over-end mixing because the exchange device was not properly sealed.	Perform binding step with gentle agitation on a plate shaker. If using end-over-end mixing, seal the vent hole in the exchange device cap with tape. Remove tape prior to centrifugation.
Sample leaked through the frit due high detergent or organic solvent concentration.	Check filtrate in 50 mL collection tube for protein. Consult the Chemical Compatibility section for appropriate buffer formulae.
Sample bound non-specifically to the device.	Check chemical compatibility of buffers used.
Protein precipitated due to over-concentration in the Amicon® Ultra-0.5 device.	Reduce duration of centrifugation time during the concentration step.
Protein was lost during sample concentration using the Amicon® Ultra-0.5 device.	Check the filtrate in the 50 mL collection tube for the protein. Verify the protein's expected molecular weight and confirm that the appropriate MWCO Amicon® Ultra-0.5 device was used.

Troubleshooting, continued

Issue: Protein purity is poor (non-specific binding)	
Possible Cause	**Solution**
Sample was degraded due to sub-optimal culture conditions.	Optimize growth/induction conditions.
Sample was degraded due to sub-optimal lysis conditions.	Optimize lysis parameters. Include protease inhibitors in lysis buffers.
Cell lysate was too concentrated.	Dilute lysate in binding buffer.
Cell lysate was too viscous.	Dilute lysate in binding buffer. Include Benzonase® nuclease in lysis buffer to remove free RNA/DNA.
Ratio of sample to resin was incorrect.	Verify the level of target protein present in the lysate and the binding capacity of the resin, and optimize the ratio.
Target protein did not bind well to resin.	Optimize the buffer conditions during binding.
Insufficient washing.	Increase volume of wash buffer or number of wash steps. Supplement the wash buffer with detergents.
Resin demonstrated high non-specific binding of proteins in addition to the target.	Optimize the buffer conditions during binding, increase the stringency of the wash buffer, or increase the volume of wash buffer or number of wash steps.
Non-specific proteins bound directly to target protein.	Add β-mercaptoethanol (up to 20 mM) to reduce sulfide bonds. Add detergents to disrupt non-specific interactions.
Issue: Entire sample volume does not flow through the device	
Possible Cause	**Solution**
Packed resin volume of ≥ 500 µL was used.	During wash and elution steps, centrifuge at 2,000 × g for 4 minutes to ensure filtrate clearance.
Sample volume did not pass through the Amicon® Ultra-0.5 device.	Increase the centrifugation time and/or confirm g-force used. 4,000 × g is recommended.
Sample volume was too large for the Amicon® Ultra-0.5 device to process.	Increase the centrifugation time or process the volume in smaller batches.

Specifications

Amicon® Pro Purification Device

Maximum volume	10 mL
Recommended relative centrifugal force	1,000 × g for flow-through, wash, and elution steps 4,000 × g for concentration/buffer exchange
Maximum relative centrifugal force	4,000 × g
Centrifuge compatibility	Swinging-bucket rotor only
Exchange device hold-up volume	< 1 µL after expelling residual volume with exchange cap

Amicon® Ultra-0.5 Device

Maximum initial sample volume	0.5 mL*
Typical final concentrate volume	15–20 µL
Recommended relative centrifugal force	14,000 × g for concentration/buffer exchange (when used as a stand-alone component) 1,000 × g for recovery (reverse) spin
Maximum relative centrifugal force	15,000 × g (when used as a stand-alone component)
Centrifuge compatibility	Swinging-bucket rotor when coupled to Amicon® Pro exchange device, or microcentrifuge fixed-angle rotor when spun with Amicon® Ultra-0.5 collection tube.
Active membrane area	1 cm^2
Hold-up volume	< 5 µL

Dimensions

Amicon® Pro collection tube plus cap	Length: 120 mm (4.7 in.)	Diameter: 34.3 mm (1.35 in.)
Amicon® Ultra device alone	Length: 29.5 mm (1.2 in.)	Diameter: 9.4 mm (0.4 in.)
Amicon® Ultra device and tube (recovery mode)	Length: 47.4 mm (1.9 in.)	
Amicon® Ultra collection tube (cap closed)	Length: 42.1 mm (1.7 in.)	Diameter: 10.8 mm (0.4 in.)

Materials of Construction

Amicon® Pro collection tube	Polypropylene
Amicon® Pro collection tube cap	Polyethylene
Amicon® Pro holder tube	Polypropylene
Amicon® Pro exchange device	Polypropylene, thermoplastic elastomer (TPE)
Frit	Polypropylene/polyethylene
Amicon® Ultra-0.5 device	Copolymer styrene/butadiene
Amicon® Ultra-0.5 collection tube	Polypropylene
Amicon® Ultra-0.5 membrane	Ultracel® low binding regenerated cellulose

* Under most circumstances, samples will not be loaded directly into the Amicon®-Ultra-0.5 device. Sample concentration will occur simultaneously with protein elution or buffer exchange and will involve loading sample (or buffer) into the exchange device.

Chemical Compatibility

The Amicon® Pro Purification System is intended for use with biological fluids and aqueous solutions. Before use, check the sample for chemical compatibility with the device.

Table 3. Chemical Compatibility of the Amicon® Pro Purification System

Acids	Concentration		Concentration
Acetic acid	≤ 50%*	Phosphoric acid	≤ 30%
Formic acid	≤ 5%*	Sulfamic acid	≤ 3%
Hydrochloric acid	≤ 1.0 M	Sulfuric acid	≤ 3%
Lactic acid	≤ 50%	Trichloroacetic acid (TCA)	≤ 10%*
Nitric acid	≤ 10%	Trifluoroacetic acid (TFA)	≤ 30%*
Alkalis			
Ammonium hydroxide	≤ 10%	Sodium hydroxide	≤ 0.5 M
Alcohols			
n-Butanol	≤ 70%	Isopropanol	≤ 70%
Ethanol	≤ 70%	Methanol	≤ 60%
Detergents			
Alconox® detergent	≤ 1%	Sodium dodecyl sulfate (SDS)	≤ 0.1%
CHAPS detergent	≤ 0.1%	Tergazyme® detergent	≤ 1%
Lubrol® PX detergent	≤ 0.1%	Triton® X-100 surfactant	≤ 0.1%
Nonidet™ P-40 surfactant	≤ 2%	Tween® 20 surfactant	≤ 0.1%
Sodium deoxycholate	≤ 5%		
Organic solvents			
Acetone	not recommended	Ethyl acetate	not recommended
Acetonitrile	≤ 20%	Formaldehyde	≤ 5%
Benzene	not recommended	Pyridine	not recommended
Carbon tetrachloride	not recommended	Tetrahydrofuran	not recommended
Chloroform	not recommended	Toluene	not recommended
Dimethyl sulfoxide (DMSO)	≤ 5%*		
Miscellaneous			
Ammonium sulfate	saturated	Phenol	≤ 1%
Diethyl pyrocarbonate	≤ 0.2%	Phosphate buffer (pH 8.2)	≤ 1 M
Dithiothreitol (DTT)	≤ 0.1 M	Polyethylene glycol	≤ 10%
Glycerine	≤ 70%	Sodium carbonate	≤ 20%
Guanidine HCl	≤ 6 M	Tris buffer (pH 8.2)	≤ 1 M
Imidazole	≤ 100 mM	Urea	≤ 8 M
Mercaptoethanol	≤ 0.1 M		

* Contact with this chemical may cause materials to leach out of the component parts. Solvent blanks are recommended to determine whether leachables represent potential assay interferences

Ordering Information

This section lists catalogue numbers for Amicon® Pro Purification Systems, Kits, and associated products. See Technical Assistance section for contact information. You can purchase these products on-line at www.millipore.com/products.

Description **Catalogue Number**

Amicon® Pro Purification Systems	Amicon® Pro + Amicon® Ultra-0.5 Devices*				
	3K	10K	30K	50K	100K
Amicon® Pro Purification System (12/pk)	ACS500312	ACS501012	ACS503012	ACS505012	ACS510012
Amicon® Pro Purification System (24/pk)	ACS500324	ACS501024	ACS503024	ACS505024	ACS510024

Amicon® Pro Purification Kits (12/pk)	Reagent Kit + Amicon® Pro Purification System*					Reagent Kit (Resin and buffers only; no devices)
	3K	10K	30K	50K	100K	
Amicon® Pro Affinity Concentration Kit Ni-NTA	ACK5003NT	ACK5010NT	ACK5030NT	ACK5050NT	ACK5100NT	ACR5000NT
Amicon® Pro Affinity Concentration Kit GST	ACK5003GS	ACK5010GS	ACK5030GS	ACK5050GS	ACK5100GS	ACR5000GS
Amicon® Pro Affinity Concentration Kit Protein A	ACK5003PA	ACK5010PA	ACK5030PA	ACK5050PA	ACK5100PA	ACR5000PA
Amicon® Pro Affinity Concentration Kit Protein G	ACK5003PG	ACK5010PG	ACK5030PG	ACK5050PG	ACK5100PG	ACR5000PG

* Choose cat. no. by Amicon® Ultra-0.5 device MWCO

Amicon® Pro Device (24/pk)	
Includes collection tube and cap, exchange device, and holder tube. Does **not** include Amicon® Ultra-0.5 filters.	ACS500024

Ordering Information, continued

Additional Reagents**	Cat. No.	Qty
Ni-NTA His•Bind® Resin	70666-3 70666-4 70666-5	10 mL 25 mL 100 mL
Ni-NTA Buffer Kit	70899	1 kit
GST•Bind™ Resin	70541-3 70541-4 70541-5	10 mL 50 mL 25 mL
GST•Bind™ Buffer Kit	70534	1 kit
Protein A Agarose	16-125	10 mL
Protein G Agarose	16-266	10 mL

** For more information on agarose resins, including binding capacity, go to www.millipore.com and enter the resin catalogue number in the search window.

Technical Assistance

Visit the tech service page on our web site at www.millipore.com/techservice.

Standard Warranty

The applicable warranty for the products listed in this publication may be found at www.millipore.com/terms ("Conditions of Sale").