EMD MILLIPORE

Purify biologically active proteins with gentle, all-in-one recovery.

Amicon® Pro Purification System

Fisher Scientific

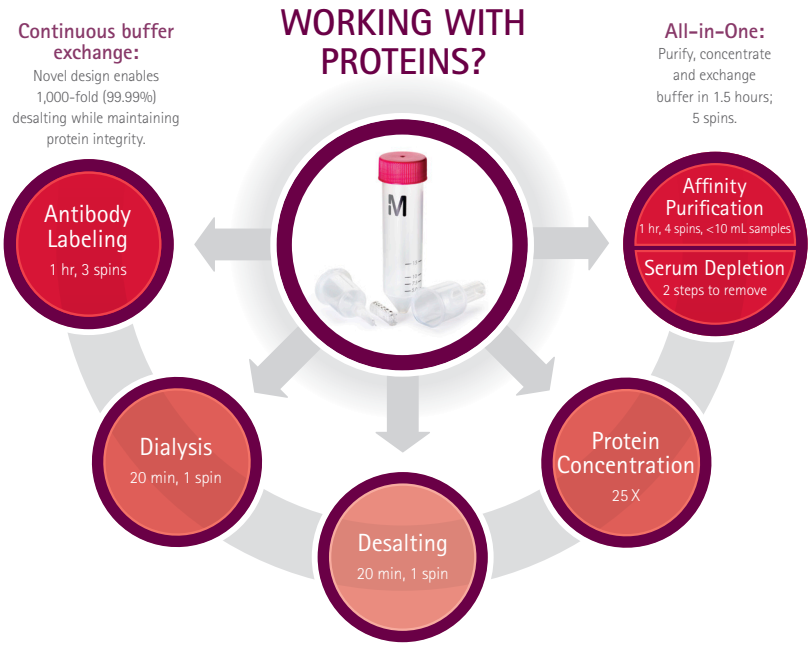
EMD Millipore is a division of Merck KGaA, Darmstadt, Germany

Biologically active proteins yield meaningful data.

When you start with consistent yields of active, native-folded protein, you're giving your experiment the best chance to succeed. If your current protein preps involve juggling columns, dialyzers and multiple transfer steps, you could be introducing variability into your data. For your next protein preparation, choose the simple, gentle method that tackles even the most labile and poorly expressed proteins—the Amicon® Pro purification system. **When your proteins behave, your research will flourish.**

"Using the Amicon® Pro format reduces our environmental footprint and simplifies the workup of our cell-free protein synthesis reactions."

Anders Pederson
University of Gothenburg



A simple, flexible tool for the basic researcher.

Whether you're performing affinity purification on a precious sample, labeling antibodies, depleting abundant proteins from serum samples or removing salts from a chromatography sample, the Amicon® Pro system is your sample preparation partner. The modular design not only allows flexibility in application, but offers unprecedented simplicity in protein sample preparation.

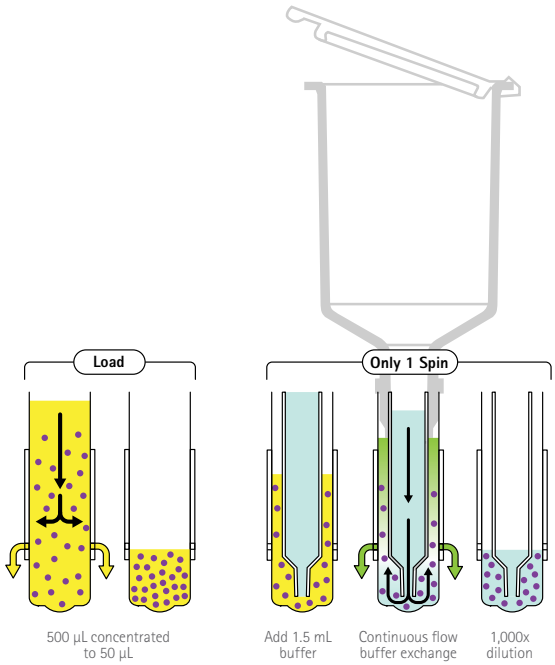
Examples:

- Turn your crude lysate into a purified, concentrated protein ready for your downstream assay in as few as four spins.
- Perform a 99% buffer exchange in a patent-pending, continuous, gentle process in one spin.

Novel engineering provides unmatched buffer exchange

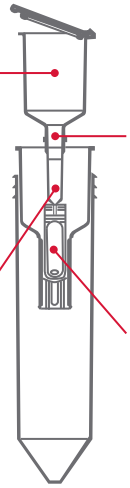
The Amicon® Pro device is the first of its kind to offer dynamic, continuous buffer exchange by diafiltration.

How does it work? The secret is in the design of the Amicon® Pro exchange device and tip. The lower portion of the exchange device is designed to exactly match the contours of the Amicon® Ultra-0.5 mL filter. The tip is tapered to maximize the external-to-internal volume ratio, ensuring that fresh buffer is slowly but consistently metered in, mixed with sample, and forced across the membrane and out. This delivers a continuous, controlled flow during desalting and buffer exchange, without multiple dilute-and-concentrate centrifugation steps. The results are **the gentle recovery of greater than 95% of purified protein.**



1,000-fold buffer exchange in a single spin. Protein (purple dots) is dissolved in Buffer 1 (yellow) during the concentration step (left). During the buffer exchange step, 1.5 mL of Buffer 2 (blue) is added. Arrows indicate direction of fluid flow, showing that Buffer 2 enters the sample continuously while Buffer 1-containing solution is being forced out.

Amicon® Pro system unique design features and workflow benefits



Exchange Device

Just one spin required with the large 10 mL chamber for the pre-wash, bind, wash, elute and buffer exchange steps

Exchange Tip

Continuous buffer exchange achieved with the unique exchange tip design

Frit

All-in-One device achieved with the frit holding back the affinity purification resin

Amicon® Ultra 0.5 mL Filter

Gentle elution and concentration of your protein sample in a single spin enabled by the Amicon® Ultra 0.5 mL filter

"If I was doing things the old way I would be six months – if not a year – behind where I am right now with my project."

Jason Lehmann
University of California in San Diego

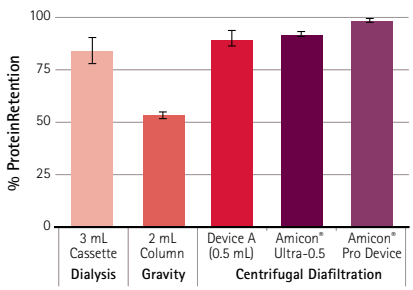
"We can now ask more questions using our final product."

Dr. Charis Pericleous
University College London

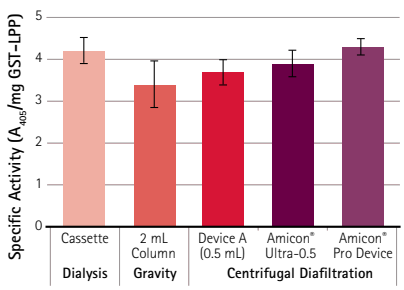
What more will you discover using the Amicon® Pro system?

Don't lose protein in multiple devices. Maximize your protein recovery.

Traditional protein purification can be a long process with multiple steps and devices, which can often result in protein degradation and loss along the way. By using the Amicon® Pro Purification System, you can avoid the risks involved with sample transfer and you can reduce hands-on time. Whether you need to affinity purify, concentrate, dialyze or any combination of the three, the Amicon® Pro Purification System will save you time and improve your protein recovery. It can help you perform multiple protein preparations in parallel, improving prep-to-prep reproducibility and enabling head-to-head comparison of expression constructs.



Higher protein retention. Compared to other buffer exchange methods tested, the Amicon® Pro device retained the highest percentage of bovine serum albumin (n=3, error bars represent standard deviation).



Higher specific activity. Compared to other buffer exchange methods, the Amicon® Pro device yielded protein with equivalent specific activity to dialysis (n=3). Results demonstrate the negative impact of multiple dilution/concentration cycles. Error bars represent standard deviation.

Amicon® Pro Device Quick Protocol Guide

NOTE: For all protocols using the Amicon® Pro device, all steps, with the exception of binding reactions, are spin-based. A swinging bucket rotor is required for all steps with the exception of the reverse spin.

Recombinant protein purification (GST or His-Tag; 0.5 mL lysate)

1. Add 200 µL resin* (50% slurry) and 1.5 mL Wash Buffer to exchange reservoir. Spin 1,000 x g for 1 min.
2. Add 0.5 mL sample and mix with resin by pipetting. Incubate with gentle agitation for 1 h.
3. Spin 1,000 x g for 1 min to clear unbound species.
4. Add 1.5 mL Wash Buffer. Spin 1,000 x g for 1 min.
5. Attach the Amicon® Ultra 0.5 mL filter (10k MWCO).
6. Add 1 mL Elution Buffer and mix with resin. Incubate for 5 min.
7. Spin 4,000 x g for 15 min.
8. Add 1.5 mL desired buffer. Spin 4,000 x g for 15 min to exchange buffer and concentrate.
9. Recover purified protein from the Amicon® Ultra 0.5 mL filter by reverse spin.

*This protocol is limited by the processing capacity of the Amicon® Ultra-0.5 mL device which is ~ 1 mg protein. Larger volumes where the target is more dilute may be processed, although reaction parameter (reagent volumes, spin durations) will need to be optimized.

To learn more, please visit: www.fishersci.com/amicon

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Antibody purification using protein A/G agarose resins (0.5 mL sample)

1. Add 200 µL resin* (50% slurry) and 1.5 mL Wash Buffer to exchange reservoir. Spin 1,000 x g for 1 min.
2. Add 0.5 mL sample and mix with resin by pipetting. Incubate with gentle agitation for 1 h.
3. Spin 1,000 x g for 1 min to clear unbound species.
4. Add 1.5 mL Wash Buffer. Spin 1,000 x g for 1 min.
5. Attach the Amicon® Ultra 0.5 mL filter (10k MWCO).
6. Add 1 mL Elution Buffer and mix with resin. Spin 4,000 x g for 10 min.
7. Add 75 µL Neutralization Buffer. Spin 4,000 x g for 5 min.
8. Add 1.5 mL final buffer. Spin 4,000 x g for 15 min to exchange buffer and concentrate.
9. Recover purified antibody from the Amicon® Ultra 0.5 mL filter by reverse spin.

*This protocol is limited by the processing capacity of the Amicon® Ultra-0.5 mL device which is ~ 1 mg protein. Larger volumes where the target is more dilute may be processed, although reaction parameter (reagent volumes, spin durations) will need to be optimized.

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Affinity purification without buffer exchange and concentration

1. Add up to 1,000* µL of packed resin and up to 9 mL Wash Buffer. Spin 1,000 x g for 1 min to clear storage buffer. For ≥ 500 µL packed resin, spin 2,000 x g for 4 min.
2. Add up to 9* mL sample and mix with resin by pipeting. Incubate with gentle agitation for 1 h*.
3. Spin to clear unbound species: For reactions using < 500 µL of packed resin, spin 1,000 x g for 1 min. For reactions using ≥ 500 µL of packed resin, spin 2,000 x g for 4 min.
4. Add Wash Buffer (using 7.5-times the packed resin volume) and spin: For reactions using < 500 µL of packed resin, spin 1,000 x g for 1 min. For reactions using ≥ 500 µL of packed resin, spin 2,000 x g for 4 mins.
5. Transfer Amicon® Pro device to a clean 50 mL collection tube
6. Add Elution Buffer (using 5 times the packed resin volume) and mix with resin. For reactions using < 500 µL of packed resin, spin 1,000 x g for 1 min. For reactions using ≥ 500 µL of packed resin, spin 2,000 x g for 4 min.
7. Recover purified sample from the collection tube.
8. For Antibody (Ig) Purification, add Neutralization Buffer to final sample.

*For large 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).

To order the Amicon® Pro Purification System without the Amicon® Ultra 0.5 mL filter, use catalogue number ACS500024.

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Amicon® Pro Device Quick Protocol Guide

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Desalting or buffer exchange with concentration

1. Pre-wet device using 0.5 mL TBS-T. Spin 1,000 x g for 1 min.
2. Optional Wash: Add 1.5 mL appropriate buffer. Spin 1,000 x g for 1 min.
3. Attach an Amicon® Ultra 0.5 mL filter (five MWCO options available) to the exchange device.
4. Add 1.5 mL* of sample to the exchange reservoir. Spin 4,000 x g for 15 min to concentrate.
5. Add 1.5 mL* of desired buffer. Spin 4,000 x g for 15 min to exchange buffer and concentrate.
6. Recover purified protein from the Amicon® Ultra 0.5 mL filter by reverse spin.

*For larger sample/buffer volumes, spin time will need to be increased appropriately. Please consult user guide for specific instructions.

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Amicon® Pro Device Quick Protocol Guide

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Antibody labeling (25–200 µg)

1. Pre-wet device using 0.5 mL TBS-T. Spin 1,000 x g for 1 min.
2. Load antibody sample into device
   - **For dilute antibodies (< 1 mg/mL)** – attach an Amicon® Ultra 0.5 mL filter (10k MWCO) to the base of the Amicon® Pro device.
     - Load antibody (up to 1 mL) into the exchange device. Spin 4,000 x g for 15 min.
   - **For concentrated Abs (≥ 1 mg/mL)** - load sample (up to 100 µL) into an Amicon® Ultra 0.5 mL filter (10k MWCO).
     - Attach filter to the base of the Amicon® Pro device.
3. Prepare a 1.5 mL reaction cocktail containing dye in appropriate reaction buffer. Add cocktail to the exchange reservoir.
4. Spin 4,000 x g for 15 min.
5. Optional: Incubate sample for an additional 30 minutes at room temperature.
6. Add 1.5 mL phosphate-buffered saline (PBS) ± Na Azide to the exchange reservoir.
7. Spin 4,000 x g for 15 min to exchange buffer and concentrate.
8. Recover labeled antibody from the Amicon® Ultra 0.5 mL filter by reverse spin.

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Ordering Information

To choose the appropriate Amicon® Pro device, determine the molecular weight cut-off (MWCO) of your protein of interest and your desired affinity purification scheme. For convenience and ease of use, the Amicon® Pro purification kits contain devices, reagents and buffers optimized for twelve reactions. These kits are ideal for affinity purification of tagged recombinant proteins, antibody purification and depletion.

Amicon® Pro Purification Kits 12/pk Includes reagent kit (resin & buffers)	Reagent Kit Only	MWCO				
		3,000	10,000	30,000	50,000	100,000
Amicon® Pro Affinity Concentration Kit Ni-NTA	ACR5000NT	ACK5003NT	ACK5010NT	ACK5030NT	ACK5050NT	ACK5100NT
Amicon® Pro Affinity Concentration Kit Protein A	ACR5000PA	ACK5003PA	ACK5010PA	ACK5030PA	ACK5050PA	ACK5100PA
Amicon® Pro Affinity Concentration Kit Protein G	ACR5000PG	ACK5003PG	ACK5010PG	ACK5030PG	ACK5050PG	ACK5100PG
Amicon® Pro Affinity Concentration Kit GST	ACR5000GS	ACK5003GS	ACK5010GS	ACK5030GS	ACK5050GS	ACK5100GS

Amicon® Pro Purification System – No Reagents Included	MWCO				
	3,000	10,000	30,000	50,000	100,000
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 System 24/pk without Amicon® Ultra 0.5 mL filter: ACS500024

What more will you discover using the Amicon® Pro system? Find out at:
www.fishersci.com/amicon

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Fisher Lit. No. BN0815142 BS GEN-14-10290 08/2014 Printed in the USA.
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