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Amicon NMWCO Filter Concentration and Buffer Exchange

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ABSTRACT

This Amicon NMWCO filter protocol helps concentration dilute elution fractions containing protein as well as assists with buffer exchange if necessary.

PROTOCOL CITATION

Lauren Adams 2022. Amicon NMWCO Filter Concentration and Buffer Exchange. **protocols.io** https://protocols.io/view/amicon-nmwco-filter-concentration-and-buffer-excha-cbwvspe6

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MATERIALS TEXT

Amicon NMWCO Filter (MWCO dependent on the protein target)
Centrifuge
0.5% TFA or 0.2% FA in mass spec grade H2O
LoBind microcentrifuge tubes

1 Wash the filter three times with Optima grade water and centrifuging at 14,000 x g. Equilibrate the column by washing once with the same buffer as the elution buffer containing the target protein for ~10-30 minutes or until the volume has decreased.

2 Load the elution fraction containing the target protein into the filter and centrifuge at 14,000 x g for ~10-30 minutes or until the volume has decreased. Repeat this until the total elution volume has decreased ≤ 25 µL for LC-MS or ≤ 80 µL for I2MS. If the buffer is already acidic (TFA or FA), no additional buffer exchange is required. However, if the elution buffer is not acidic (ex: 100 mM Tris Base), buffer exchange should be performed.

3 Gradually incorporate larger volumes of 0.2-0.5% TFA or FA to the sample volume after sequential centrifuge cycles until the majority of the final buffer is TFA or FA. Remove sample from filter by centrifuging upside down at 14,000 x g into a clean LoBind tube. Bring the final volume up to 25 µL for LC-MS or 80 µL for I2MS with 0.2-0.5% TFA or FA before analyzing by mass spectrometry.