Application Note

INTEGRA

Protein purification using immobilized metal affinity chromatography (IMAC) with a VIAFLO 96/384 handheld electronic pipette

Introduction

Immobilized metal affinity chromatography (IMAC) is a method used to purify tagged proteins according to their affinity to specific metal ions. The experimental procedure involves many pipetting steps that are challenging and time consuming for the researcher, especially when performed in a 96 well format. The VIAFLO 96/384 electronic multichannel pipette offers a unique solution for this protocol, allowing simultaneous pipetting of 96 samples with the ease of use of a traditional handheld pipette, to significantly increase the sample throughput and enhance data reproducibility. Its compact design means that the VIAFLO 96/384 system requires little space and fits easily on the workbench.

Key benefits:

- Samples and buffers are simultaneously transferred into a 96 well plate, increasing the purification throughput by minimizing the user's hands-on time.
- The capability to program and save the protocol on the VIAFLO 96/384 reduces user-to-user variability in the laboratory, increasing the reproducibility of results.
- The Tip Align setting automatically aligns the tips in the center of each well, supporting the user during pipetting and preventing tips from crashing into the 96 well plate.
- With its space saving footprint, the VIAFLO 96/384 handheld electronic pipette only requires a limited space on the laboratory workbench and fits into a laminar flow cabinet, allowing the user to work under sterile or controlled conditions whenever necessary.

Figure 1: VIAFLO 96/384 with a 96 channel pipetting head (50-1250 µl) and GripTip pipette tips.

Step-by-step procedure:

Experimental set-up

The following protocol shows an example set-up for performing an IMAC protein purification with a VIAFLO 96/384 handheld electronic pipette. The protocol uses 96 channel pipetting heads in the volume ranges of 50-1250 µl and 5-125 µl with Non-Sterile GripTips (**Figure 1**).

1. Culture bacteria

STEP: Culture bacteria for protein expression in 96 well format.

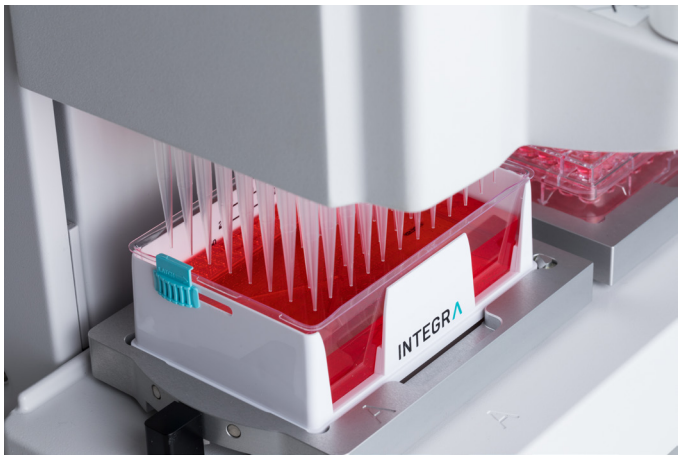

Figure 2: Example of a 300 ml automation friendly reagent reservoir placed on a VIAFLO 96/384.

HOW TO: This step uses the VIAFLO 96/384 with a 96 channel pipetting head 1250 µl and Non-Sterile GripTips.

Fill bacteria growth media into a 300 ml automation friendly reagent reservoir and place it on **Position A** of the VIAFLO 96/384 deck (**Figure 2**). Put a 2.2 ml 96 well, deep well plate on **Position B** and transfer the media into it using the VIAFLO 96/384 handheld electronic pipette in Pipet mode. Inoculate the growth media with bacteria by transferring them into the pre-filled 96 well plate. Incubate the bacteria overnight at 37 °C to express the protein of interest and then centrifuge until they form a pellet. Use the Pipet mode of the VIAFLO 96/384 to carefully aspirate the supernatant without touching the cell pellet.

Tips:

- By using the z-height limit, it is possible to fix the tips so that you can't touch the cell pellet.
- Set the Tip Align strength to 2 to allow the user to manually move the pipette tips to one side of the well to avoid disturbing the pellet during aspiration. Use a slow aspiration speed – speed 2/3 – to avoid accidentally sucking up the cell pellet.

2. Preparation of cell extract

STEP: Lysis of bacteria and lysate filtration.

HOW TO: This step uses the VIAFLO 96/384 with the 96 channel pipetting head 125 µl and Non-Sterile GripTips for the first stage of the process and then the 96 channel pipetting head 1250 µl and Non-Sterile GripTips for the lysate filtration pipetting step.

Fill lysis buffer into a 150 ml automation friendly reagent reservoir (**Position A**). Transfer the buffer onto the cell pellet (**Position B**). Use the Pipet/Mix mode of the VIAFLO 96/384 to resuspend the pellet in the lysis buffer, mixing thoroughly to ensure it is homogeneous. Incubate at room temperature for 1 hour on an orbital shaker. Prepare equilibration buffer in a 150 ml automation friendly reagent reservoir (**Position A**) and transfer it to the cell lysate. Centrifuge the plate to separate the cell debris from the supernatant containing the protein of interest.

Switch to the 96 channel pipetting head 1250 µl with Non-Sterile GripTips for the lysate filtration step. Attach a 96 well, 0.65 µm MultiScreenHTS DV Filter Plate (Merck Millipore) on top of a 1.2 ml 96 well, deep well plate and carefully transfer the supernatant using the Pipet mode of the VIAFLO 96/384. Repeat this filtration step until no supernatant remains.

Tips:
- Fix the z-height to prevent disturbance of the pellet and to avoid damaging the filter mesh.
- Aspirate at low speed – speed 1/2 – and watch the liquid level of the supernatant carefully.

Note: Filtration of the lysate can also be performed with a vacuum manifold instead of a plate centrifuge.

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3. IMAC protein purification

STEP: Prepare the HisPur™ Cobalt Spin Plate (Thermo Scientific) and transfer the cell lysate.

HOW TO: The plate preparation and protein purification steps are performed using the VIAFLO 96/384 with the 96 channel pipetting head 1250 µl, and involve several liquid transfer stages. **Position A** of the VIAFLO 96/384 deck is dedicated to 300 ml automation friendly reagent reservoirs containing the ultrapure water and equilibration, wash and elution buffers. The purification plate is placed on **Position B**.

Place the HisPur™ Cobalt Spin Plate on the collection plate provided and centrifuge to discard the preloaded storage solution. Quickly dry the collection plate by tapping it on a paper towel and then remount the purification plate. Add ultrapure water and then centrifuge to remove any residual storage buffer, then add equilibration buffer. Allow to equilibrate and then centrifuge to remove buffer. Repeat the equilibration step.

Load the filtered lysate onto the prepared plate using a low pipetting speed. Wash the plate several times to remove unspecific proteins from the sample. Finally, elute the purified protein with the elution buffer.

Note: Dry the collection plate by tapping it on a paper towel after each step to completely remove the last drops of the different buffers.

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4. Re-buffering the purified proteins

STEP: Re-buffer the purified proteins using a Zeba™ Spin Desalting Plate (Thermo Scientific).

HOW TO:
Plate preparation
The Zeba™ Spin Desalting Plate must first be equilibrated by washing four times with the desired buffer. This step uses the 96 channel pipetting head 1250 µl with the VIAFLO 96/384 handheld electronic pipette.

Place a 300 ml automation friendly reagent reservoir filled with the equilibration buffer on **Position A** and the desalting plate on **Position B**. Transfer the equilibration buffer into the plate and centrifuge. Repeat the equilibration step three times.

Final re-buffering of the purified proteins
After equilibration, the plate can be used with the protein samples. This step uses the 96 channel pipetting head 125 µl.

Stack the desalting plate on top of a new 0.5 ml 96 well, deep well plate (**Position B**). Transfer the previously purified proteins into the plate using a low aspiration speed (speed 3). Carefully touch the pipette tips to the top of the resin. Centrifuge the desalting plate to collect the final re-buffered proteins.

Remarks

Three position stage:
Improve your workflow by using the VIAFLO 96/384 together with a three position stage. **Position A** can be then dedicated to the GripTip box, **Position AB** to the buffers and **Position B** to the multiwell plate (**Figure 3**).

Automatic mode:
The Automatic mode of the VIAFLO 96/384 enables automation of pre-programmed VIALINK protocols, minimizing the user-to-pipette interaction. This is especially helpful in confined spaces, such as a laminar flow cabinet.

Partial tip load: If your experimental set-up requires purification of less than 96 samples at once, the VIAFLO 96/384 can work with any number of tips loaded. Simply adjust the number of tips needed to perform your experiment.

Conclusion

- The VIAFLO 96/384 offers scientists a unique operation concept that makes the device as easy to use as a conventional electronic pipette.
- Optimizing individual pipette settings enables accurate sample transfers without the tips touching the bottom of the plate and disturbing the cell pellet.
- Let the VIAFLO 96/384 simply guide you step by step through the protocol, ensuring a less error-prone workflow and enhancing the reproducibility of your results.
- The VIAFLO 96/384 offers an excellent solution for multiple transfers in a 96 well format, without the user having to invest much time or effort.
- The VIAFLO 96/384 significantly increases sample throughput and decreases the user's hands-on time.

VIAFLO 96/384

Three position stage

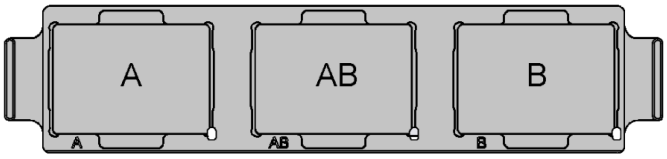

Figure 3: VIAFLO 96/384 with a three position stage.

Materials

Manufacturer	Part Number	Description	Link
INTEGRA Biosciences	6001/6031	VIAFLO 96 or 384 handheld electronic pipette (base unit)	https://www.integra-biosciences.com/global/en/electronic-pipettes/viaflo-96384#downloads
INTEGRA Biosciences	6230	Three position stage for 96 and 384 well plates	https://www.integra-biosciences.com/global/en/electronic-pipettes/viaflo-96384#parts-and-numbers
INTEGRA Biosciences	6123	96 pipetting head 125 µl	https://www.integra-biosciences.com/global/en/electronic-pipettes/viaflo-96384#parts-and-numbers
INTEGRA Biosciences	6104	96 pipetting head 1250 µl	https://www.integra-biosciences.com/switzerland/en/electronic-pipettes/viaflo-96384#parts-and-numbers
INTEGRA Biosciences	6307 6305 6327	300 ml automation friendly reagent reservoir	https://www.integra-biosciences.com/global/en/reagent-reservoirs/automation-friendly-reagent-reservoirs#parts-and-numbers
INTEGRA Biosciences	6303 6301 6317	150 ml automation friendly reagent reservoir	https://www.integra-biosciences.com/global/en/reagent-reservoirs/automation-friendly-reagent-reservoirs#parts-and-numbers
INTEGRA Biosciences	6463	125 µl Non-Sterile GripTips	https://www.integra-biosciences.com/global/en/griptip-selector-guide
INTEGRA Biosciences	6443	1250 µl Non-Sterile GripTips	https://www.integra-biosciences.com/global/en/griptip-selector-guide
Axon Lab	10018778	2.2 ml 96 well, deep well plate, PP (round bottom)	https://ch.axonlab.com/en/life-science-industry/life-science/general-lab-consumables-and-plastics/deep-well-plates/?p=1
Axon Lab	10018762	1.2 ml 96 well, deep well plate, PP (round bottom)	https://ch.axonlab.com/en/life-science-industry/life-science/general-lab-consumables-and-plastics/deep-well-plates/?p=1
Axon Lab	10018779	0.5 ml 96 well, deep well plate, PP (round bottom)	https://ch.axonlab.com/en/life-science-industry/life-science/general-lab-consumables-and-plastics/deep-well-plates/?p=1
Thermo Fisher Scientific	AB0718	Gas permeable adhesive seals	https://www.thermofisher.com/order/catalog/product/AB0580#/AB0580
Merck Millipore	MSDVN6510	MultiScreen$_{HTS}$ DV Filter Plate, 0.65 µm, clear, non-sterile	http://www.merckmillipore.com/CH/de/product/MultiScreenHTS-DV-Filter-Plate-0.65m-clear-non-sterile,MM_NF-MSDVN6510?ReferrerURL=https%3A%2F%2Fwww.google.com%2F
Thermo Fisher Scientific	90095	HisPur™ Cobalt Spin Plate	https://www.thermofisher.com/order/catalog/product/90095?SID=srch-hj-90095#/90095?SID=srch-hj-90095
Thermo Fisher Scientific	89807 89808	Zeba™ Spin Desalting Plate	https://www.thermofisher.com/order/catalog/product/89807?SID=srch-hj-89807#/89807?SID=srch-hj-89807

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