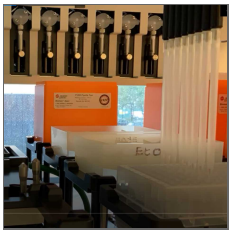

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Protein aggregation capture (PAC) and minimal automated processing for proteomics using magnetic beads and a Beckman Biomek™ NxP workstation for 96 well plates.

In 3 collections

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ABSTRACT

Sample preparation for mass spectrometry analysis involves numerous liquid transfer steps.

These include
- sample lysis,
- protein extraction,
- solubilisation,
- estimation,
- reduction and alkylation,
- normalisation,
- clean-up,
- enzymatic digestion,
- and desalting.

Adapting these steps onto an automated workstation can increase efficiency, throughput, and reduce coefficients of variance (%CV) thereby providing reliable reproducible data for statistical comparisons.

This protocol is part of a modular collection for the processing of biological samples for proteomics.

GUIDELINES

A Beckman Biomek NxP with Span-8 pod and associated software is used in this method.
Of course, alternative liquid handlers can be used with **appropriate method development**.

The Biomek is a versatile liquid handler, but this means that alternative deck orientations and system components are possible. You may need to **modify the method file** for your specific Biomek liquid handler system.

pH: PAC works in the pH range of pH 7.0 to pH 8.5 .

Protein samples should be ultrasonicated to **remove nucleic acid**. DNA if in sample will coat the PAC beads, causing their aggregation, and is best avoided. If ultrasonication is not available, use Benzonase to shear DNA. If following the protocols in this collection, this should not be an issue since ultrasonication is the entry point.

Bead concentration during binding: A bead to protein ratio of 5:1 to 10:1 is recommended. For example, if processing 20 µg of protein, add 4 µg to 2 µg of beads.

Sample concentration: The binding capacity of PAC beads provides a flexible clean-up format across a range of protein and peptide concentrations (10 µg/mL to 5 mg/mL), as long as the concentration of beads is adjusted as described above.

MATERIALS

Seq Grade Modified Trypsin, 100ug (5 x 20ug) **Promega Catalog #V5111**
multichannel pipette (300 µL)
MagReSyn Hydroxyl magnetic Beads Catalog #MR-HYX010

HEPES **Fisher Scientific Catalog #BP310** [M] 100 millimolar (mM) pH 7.5
Ammonium bicarbonate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #09830-500G** [M] 50 millimolar (mM)
(make up fresh)
Plate sealing film

[M] 0.1 % volume formic acid

Equipment

ThermoMixer® C	NAME
Eppendorf	BRAND
Catalog No. 2231000680	SKU
https://online-shop.eppendorf.us/US-en/Temperature-Control-and-Mixing-44518/Instruments-44519/Eppendorf-ThermoMixerC-PF-19703.html	LINK

Equipment

P1000	NAME
pipette tips	TYPE
Beckman Coulter	BRAND
B01124	SKU

Equipment

	NAME
Beckman Coulter	BRAND
Biomek NXp	SKU

Equipment

Full reservoir	NAME
reservoir	TYPE
Beckman	BRAND
372784	SKU

SAFETY WARNINGS

Wear PPE when operating.
Prepare solvents in a fume hood.
Store organic solvents in a flammable storage cabinet when not in use.
Discard used solvents and buffers in appropriate waste containers

BEFORE START INSTRUCTIONS

Bead preparation:
Resyn hydroxyl beads are shipped at [M] 20 µg/µL concentration in water with 0.05% sodium azide. It is a good idea to aliquot them for long term storage at 4 °C. Preparing aliquots of stock beads avoids excess handling of the main bottles and minimizes the risk of contamination.

To do this: Let stock beads equilibrate to room temperature for 30 minutes. If the beads have settled during storage they should be resuspended by inversion or gentle vortexing until no solid bead mass is visible at the bottom of the bottle. Aliquot into 10 mg , 20 mg , and 40 mg amounts, and store at 4 °C until further use.

Biomek file:
PACMethodv01.bmf299KB
This is the file to be used.

Bead preparation

4m 10s

1 Briefly vortex the beads and place the tube on a magnetic stand for 00:02:00 to collect the beads. 2m

2 Add ultrapure water at a volume corresponding to 5 to 10 times the initial volume of mixed beads. 2m 10s
Vortex the beads for 00:00:10 and place on a magnetic stand for 00:02:00 to collect the beads. Carefully aspirate and discard the wash buffer with a gel loading tip.

3 Repeat the wash steps a further two times.

4 Resuspend the beads with ultrapure water at a final concentration of [M] 20 µg/µL.

5 Washed beads may be stored at 4 °C for up to one month.

Sample plate preparation

6 To the Eppendorf 96 well / 500uL plate which contains the samples in HEPES pH 7.5 to be processed, add 5 µL of the washed beads as prepared above.
Washed beads (prepared as above) may be added to the samples in a ratio of 5-10 µg of beads to 1 µg of protein.
Check the pH of any sample, it is critical that it is in the range of Ph7.0 pH 7.0 to Ph pH 8.5 for optimal binding by measuring an aliquot on pH paper.

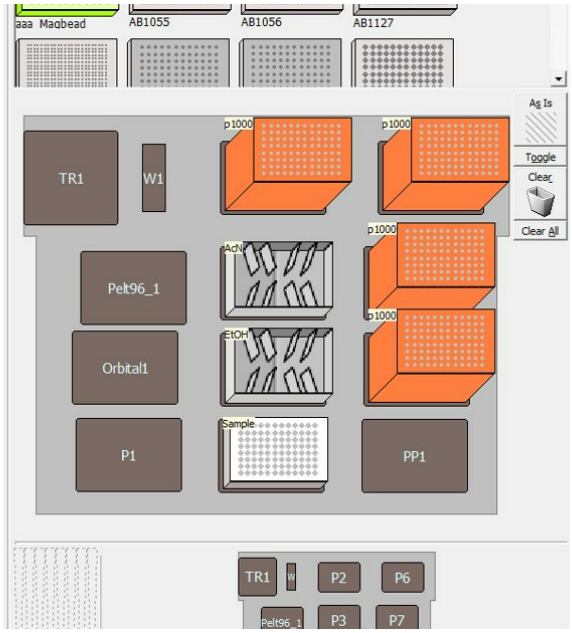
Setting up the instrument

7 Double click the software icon.

8 Under the **Method** tab, select home all axes to orient and prepare the automated liquid handler.

9 Under **File**, select **Open/Method.** Select the **PACmethodV01** method.

10 To the deck of the NxP, add four p1000 tip boxes. **SEE BELOW**
Add 1 Beckman full reservoir containing 150 mL of [M] 100 % volume acetonitrile and 1 Beckman full reservoir containing 150 mL of [M] 70 % volume acetonitrile.



deck layout

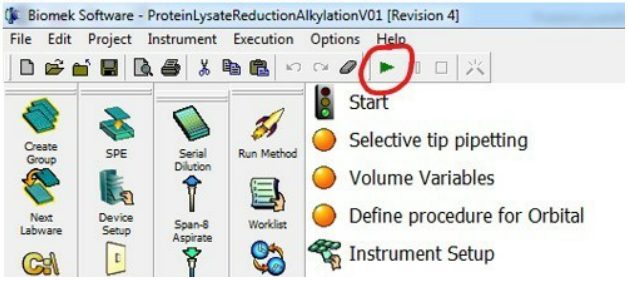
Sample Binding

11 To each of the samples in the Eppendorf 96 well 500uL plate with the Resyn beads, add 300 µL of [M] 100 % volume acetonitrile. MIx briefly on a plate shaker, then place on the Biomek instrument.

Running the method

2m

12 Start the method by clicking the green **Run** icon.



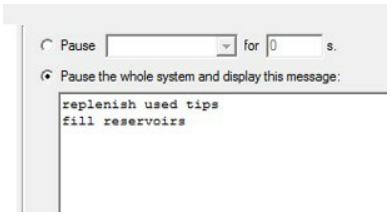
Run method

13 You will be prompted by the software to enter the location of the first column to be processed.
If your first samples are in column A1 to H1 on your sample plate, enter "**1**"
You will then be prompted to enter the value of the last column to be processed. If you have a half plate of 96 samples, enter "6".
The method will need to be modified if more samples on the plate are to be processed. This is easily done, but more pause steps will need to be built in to accommodate the extra pipette boxes needed.

14 The software will ask you to check that the deck layout matches that of the program. Once you are satisfied that this is the case, click **OK**.

Running the PAC method.

15 The workstation will pause after the acetonitrile washing steps, prompting the user to replenish used tips, and refill reservoirs if needed.



Pause

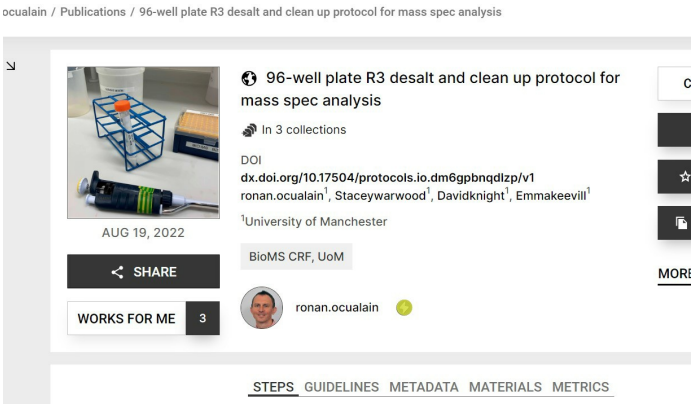
Do not reach into the workstation while the program is running, this action will break the "light curtain" and stop the system as a safety precaution.

16 After the program is complete, remove any residual liquid carefully, and add 50 µL trypsin at a **1:20** to **1:100** ratio in [M] 50 millimolar (mM) ammonium bicarbonate. Cover the plate with a sealing film, and place on a thermomixer overnight at 37 °C .

17 After digestion, remove the supernatant, leaving the beads behind.
This may be done by modifying the transfer file method, or manually by using a gel loading tip.

18 Add 100 µL of [M] 0.1 % volume formic acid to the beads, place the plate on a plate shaker for 00:02:00 , remove supernatant and pool eluates. 2m

19 Proceed to R3 desalting https://www.protocols.io/view/96-well-plate-r3-desalt-and-clean-up-protocol-for-dm6gpbnqdlzp/v1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