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🌐 Automated 96 well plate based protein reduction and alkylation using a Beckman Biomek™ NxP workstation

🔗 In 3 collections

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protocols.io Ambassadors	BioMS CRF, UoM	**1 more workspace** ↓

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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.

MATERIALS

⊗ Dithiothreitol **Fisher Scientific Catalog #BP1725**

Equipment	
96 AFA-tube TPX plate	NAME
ultrasonication plate	TYPE
Covaris	BRAND
520291	SKU

OR

Equipment	
8 AFA-Tube TPX strip	NAME
Ultrasonication strip	TYPE
Covaris	BRAND
520292	SKU

with

Equipment	
Deepwell protein lo-bind 96/500 uL plate	NAME
96 well plate	TYPE
Eppendorf	BRAND
0030504100	SKU

Equipment	
Biomek Tips P20 Sterile	NAME
tips	TYPE
Beckman	BRAND
717255	SKU

Equipment	
Biomek Tips P250 Non-Sterile	NAME
tips	TYPE
Beckman	BRAND
717251	SKU

Equipment	
8 AFA-tube TPX strip holder	NAME
holder	TYPE
Covaris	BRAND
500685	SKU

Equipment	
	NAME
Beckman Coulter	BRAND
Biomek NXp	SKU

⊗ Iodoacetamide **Merck MilliporeSigma (Sigma-Aldrich) Catalog #I1149**

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

This step should be performed after ultrasonication of samples using the Covaris LE220+ and either 96 AFA-Tube TPX plate (PN 520291), or 8 AFA -Tube TPX strip (PN 520292).

If this is not the case, place the plate containing the protein lysate to be reduced and alkylated in the Biomek workstation. For the method here, this is P1.

Method: ⬜ ProteinLysateReductionAlkylationV01.bmf 297KB

Upload and open the attached method and modify it to your system and deck configuration.

To prepare:

Prepare [M] 100 millimolar (mM) dithiothreitol (DTT) by adding ⏳ 92.4 mg to a ⏳ 15 mL Falcon tube. Add ⏳ 6 mL of milli-Q H2O, vortex mix. Store for 12 hours, then discard.

Prepare [M] 100 millimolar (mM) iodoacetamide (IAM) by adding ⏳ 55.5 mg to ⏳ 15 mL Eppendorf tube. Add ⏳ 3 mL of milli-Q H2O, vortex mix. IAM is light sensitive, cover with foil. Store for 12 hours, then discard.

Preparation

1 Double click the software icon.

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

3 Under **File**, select **Open/Method.** Select the **ProteinLysateReductionAlkylationV01** method.

4 To the deck of the NxP, add two p20 tip boxes, and 1 p250 tip box.
Add the samples in a plate to be reduced and alkylated. For the above method, this will either be a 96

AFA-Tube TPX plate, or multiples of 8 AFA-Tube TPX strips in a 8 AFA-tube TPX strip holder. If using a plate other than these, enter its details in the instrument set up step.
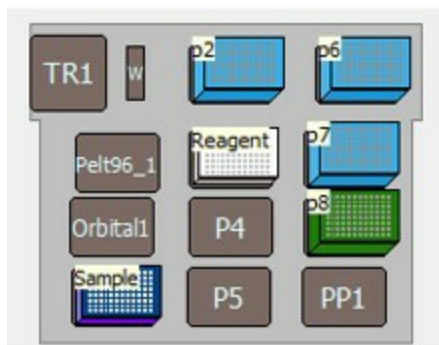
5 To a Eppendorf 96/500uL plate (reagent plate), add the following volumes into each of the 8 wells of a single column of an Eppendorf 96/500uL protein lobind deepwell plate.

- Column 1: 500 uL of 100mM DTT
- Column 2: 250 uL of 100mM IAM

Addition of 100mM DTT and IAM to the reagent plate

Setting up the deck

6 Place tip boxes, reagent, and sample plates as detailed below

⚠

Deck layout on Biomek NxP

It is important that the deck layout in the software matches that on the instrument. Mismatches can result in damage to pod.

7 The default liquid sample volume for this method is 120uL.
Volumes may be changed in plate properties
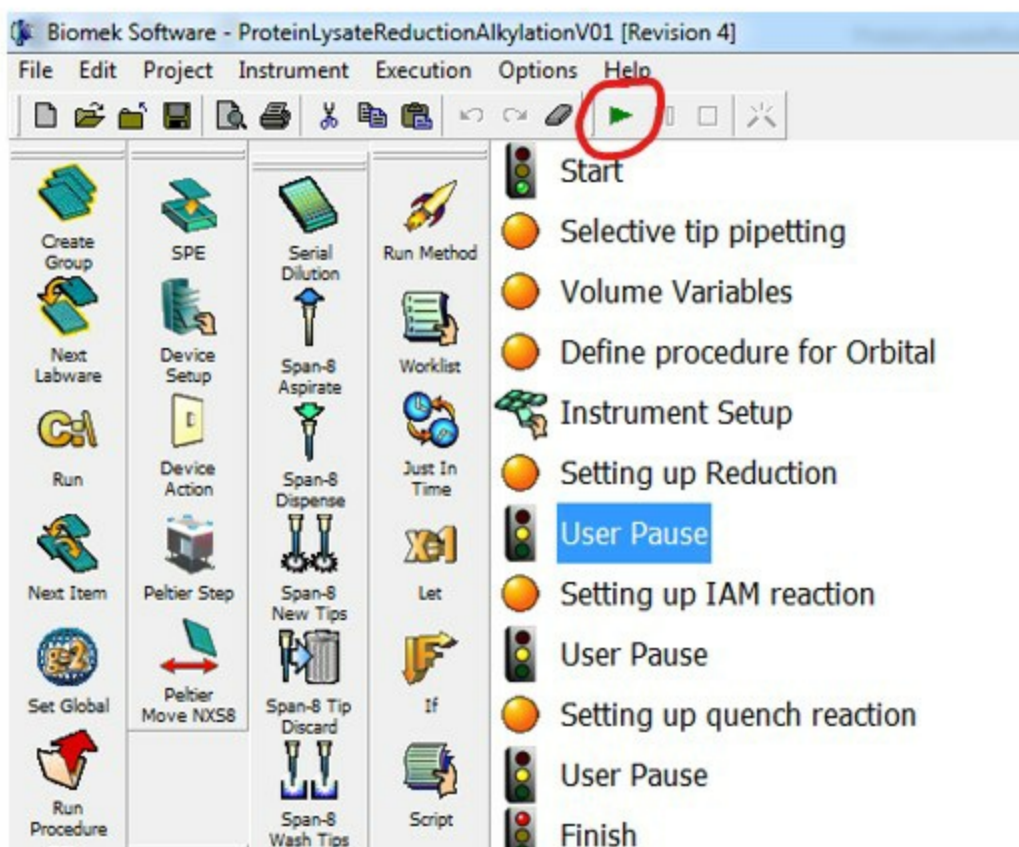

sample volumes

Starting the method

45m

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

Click **Run**

9 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 full plate of 96 samples, enter "**12**". All wells will be processed.

10 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.

The instrument will now reduce, alkylate, and quench the samples.
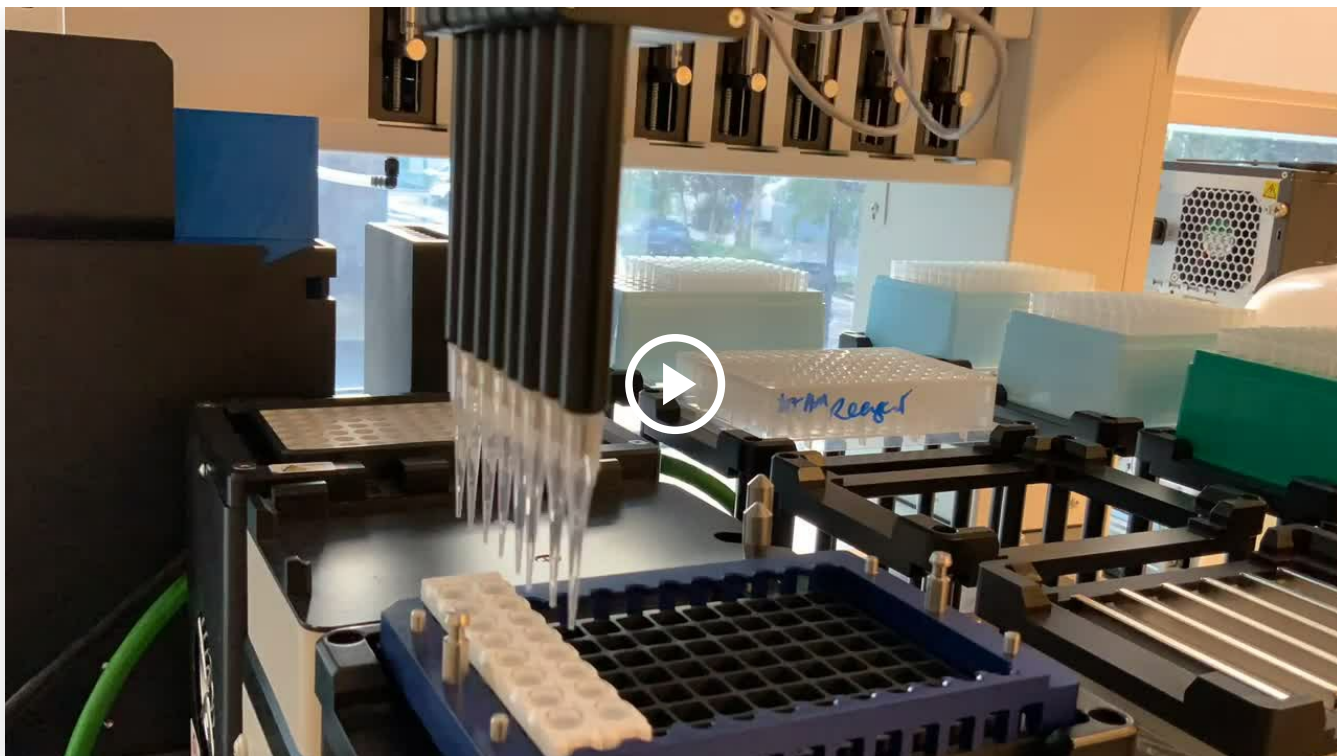
11 ⚠️ After the reduction step, the system will pause, and the user will be prompted to remove the plate and `15m` incubate it at 🌡️ 60 °C for ⏱️ 00:15:00

```
Paused
Please cover and shake samples
Incubate for 20 minutes at 60C
```

DTT pause

12 ⚠️ After the alkylation step, the system will pause, and the user will be prompted to remove the plate, cover `30m` it, and incubate it at 🌡️ Room temperature in the dark for ⏱️ 00:30:00

```
System paused - Remove plate, cover,
 and incubate in dark for 30 minutes at RT
```

IAM pause

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

14

After the program has completed, you may now proceed to protein concentration estimation, or store the samples at either 🌡 -20 °C or 🌡 -80 °C .