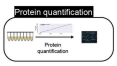
Jan 12, 2022 Version 2

Automated Protein Quantification with the Biomek-FX Liquid Handler System V.2



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Peer-reviewed method

In 1 collection

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Chen Y, Lease NK, Gin JW, Ogorzalek TL, Adams PD, Hillson NJ, Petzold CJ (2022) Modular automated bottom-up proteomic sample preparation for high-throughput applications. PLoS ONE 17(2): e0264467. doi: 10.1371/journal.pone.0264467

Protocol status: Working
We use this protocol and it's working

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Protocol Integer ID: 56561

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Abstract

This protocol details steps to perform the protein quantification (Lowry-based) assay by using a Biomek FX liquid handler system. It is optimized to assay a full 96-well plate of protein samples in duplicate with a separate (control) plate for BSA standards. You will need a plate reader to measure the samples and standards.

This protocol works best as part of a full proteomic sample preparation workflow with:

Automated Chloroform-Methanol Protein Extraction on the Biomek-FX Liquid Handler System

and

Automated Protein Normalization and Tryptic Digestion on a Biomek-FX Liquid Handler System

Image Attribution

Jonathan Vu

Guidelines

- A Beckman-Coulter Biomek FX liquid handler system with a 96-pod head is used for this protocol. Alternative liquid handlers can be used with appropriate method development.

- A Molecular Devices Spectramax 250 microplate reader is used for the protein quantification assay measurement.

- Because different deck orientations and system components are possible, you will need to modify the method file (attached in the 'Before start' section) for your specific Biomek liquid handler system.

Notes:
- This protocol is set up to measure the amount of protein in duplicate.

Materials

Hard-Shell 96-Well PCR Plates low profile thin wall skirted white/clearBIO-RADCatalog #HSP9601
Pierce Bovine Serum Albumin Standard Pre-Diluted Set Thermo Fisher, Catalog #23208
20 uL pipet tips Molecular Bioproducts BioRobotix, Catalog #918-262
200 uL pipet tips Molecular Bioproducts BioRobotix, Catalog #919-262
Corning 96 Well Black Polystyrene Microplate Fisher Scientific, Catalog #07-200-567
Reservoir Microplate Agilent, Catalog #201254-100
96 Deep Well Reagent Reservoir VWR, Catalog #101100-962
Water LC-MS grade B&J Brand VWR Scientific, Catalog #BJLC365-2.5
DC Protein Assay Reagent A by Bio-rad Laboratories, Catalog #500-0113
DC Protein Assay Reagent B by Bio-rad Laboratories, Catalog #500-0114
8-strip PCR Tubes with Caps Axygen, Catalog #14-222-251

Safety warnings

Wear proper PPE (gloves, safety goggles, and lab coat), and prepare solvents in a chemical 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

Prepare BSA Standards Plate (1st 4 rows from A to D):

1. Add 40 uL of H_2O into wells A1 to D1.
2. Add 40 uL of BSA Standards 1 (125 ug/mL) to 7 (2000 ug/mL) into columns 2 to 8.

For this protocol you will need:

1. Beckman-Coulter Biomek FX liquid handler system with a 96-pod head.
2. Upload the attached method file and modify it to fit your deck and system configuration.

Modular Protein Quantitation metho...

Deck Setup

10m

1 Open Biomek Software that controls Biomek-FX liquid handler system. Under "File" drop-down menu, click "Open" to select the automation method "Modular Protein Quantitation method."

> Note
>
> Because different deck orientations and system components are possible, you will need to modify the method file (attached in the 'Before start' section) for your specific Biomek liquid handler system.

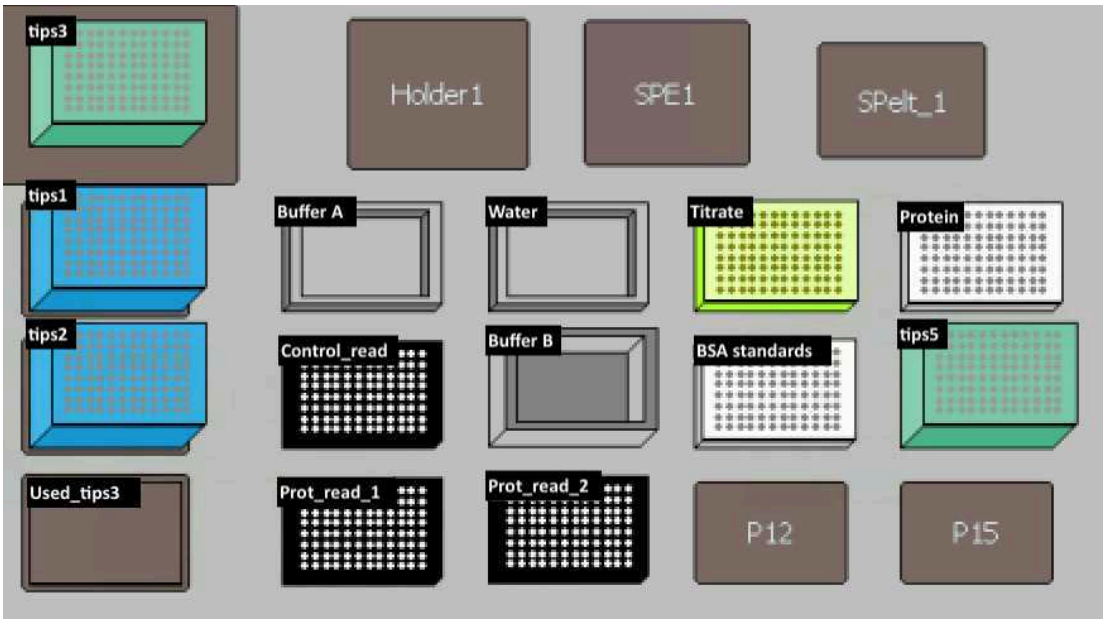
2 Click on "Instrument Setup" under the "Setup" group node to get visual instruction on how to set up the deck.

3 Set up the deck (refer to the deck setup picture below):

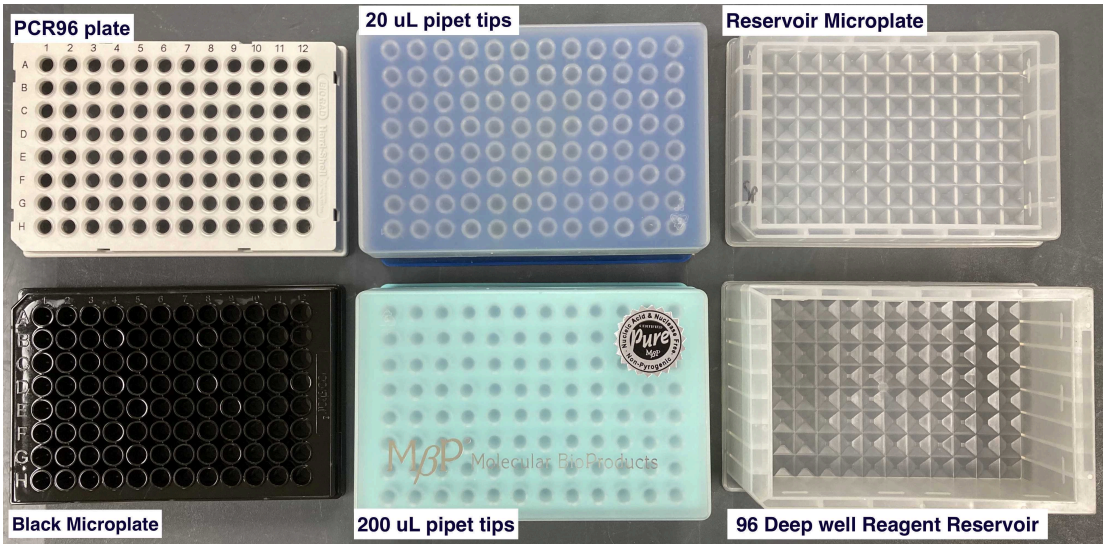
10m

	A	B	C
	Deck Label	**Labware**	**Reagent**
	protein	PCR96 plate (BIO-RAD, Cat.#HSP9601)	unknown amount of protein to quantify
	titrate	PCR96 plate (BIO-RAD, Cat.#HSP9601)	
	BSA standards	PCR96 plate	BSA Standards (Thermo Fisher, Cat.#23208)
	tips1,2	20 µl pipet tips (Molecular Bioproducts BioRobotix, Cat.#918-262)	
	tips 3,5	200 uL pipet tips (Molecular Bioproducts BioRobotix, Cat.#919-262)	
	control read, prot read 1, prot read 2	Black Microplate (Fisher Scientific, Cat.#07-200-567)	
	Buffer A	Reservoir Microplate (Agilent, Cat.#201254-100)	DC Protein Assay Reagent A (Bio-rad Laboratories, Cat.#500-0113)
	water	Reservoir Microplate (Agilent, Cat.#201254-100)	LC-MS grade Water (VWR Scientific, Cat.#BJLC365-2.5)
	Buffer B	96 Deep Well Reagent Reservoir (VWR, Cat.#101100-962)	DC Protein Assay Reagent B (Bio-rad Laboratories, Cat.#500-0114)

Materials for Deck setup



Deck setup



Labware for Deck setup

4 MANUAL STEP: Use a multichannel pipette to mix protein samples right before starting. 2m

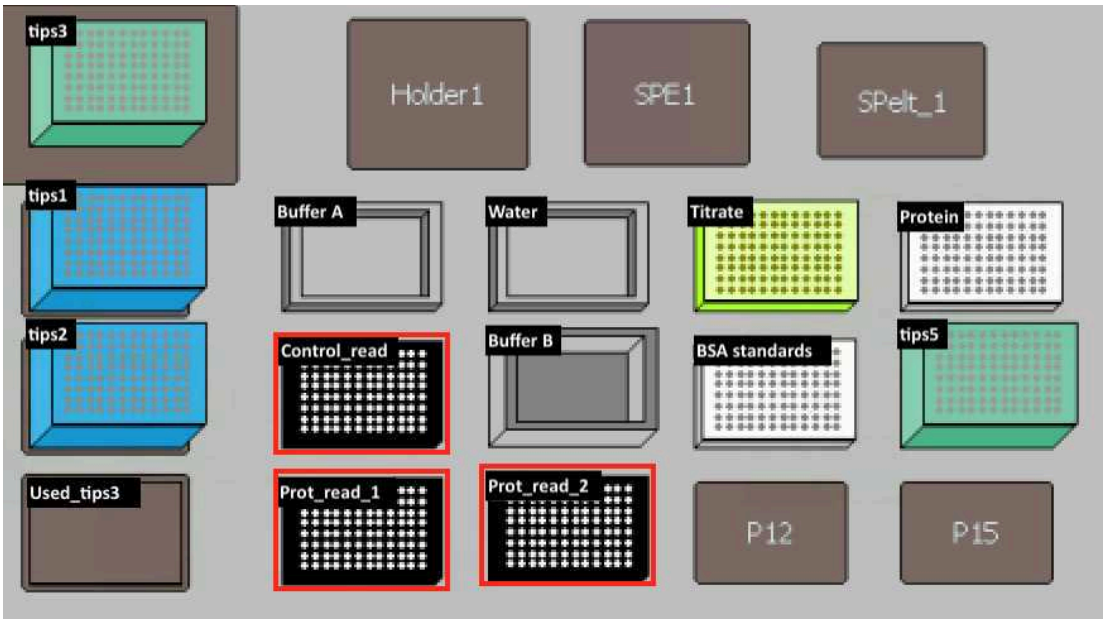
5 Click the "Run" button (green arrow) to start.

DC protein assay

25m

6 Transfer 25 µl of Buffer A to Protein Read plate 1, Protein Read plate 2, and Control Read plate.

1m

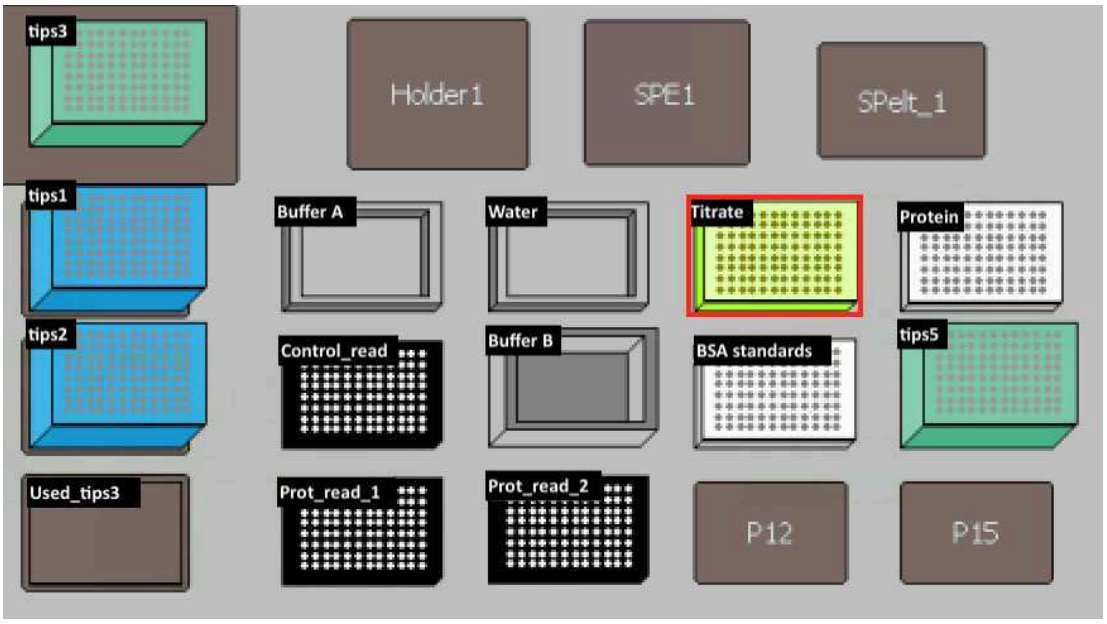


7 Transfer 12 µl of H_2O into Titrate plate. Then transfer 3 µl (see Note for more details) from Protein plate to Titrate plate and mix with 5 cycles of pipetting mixing on deck.

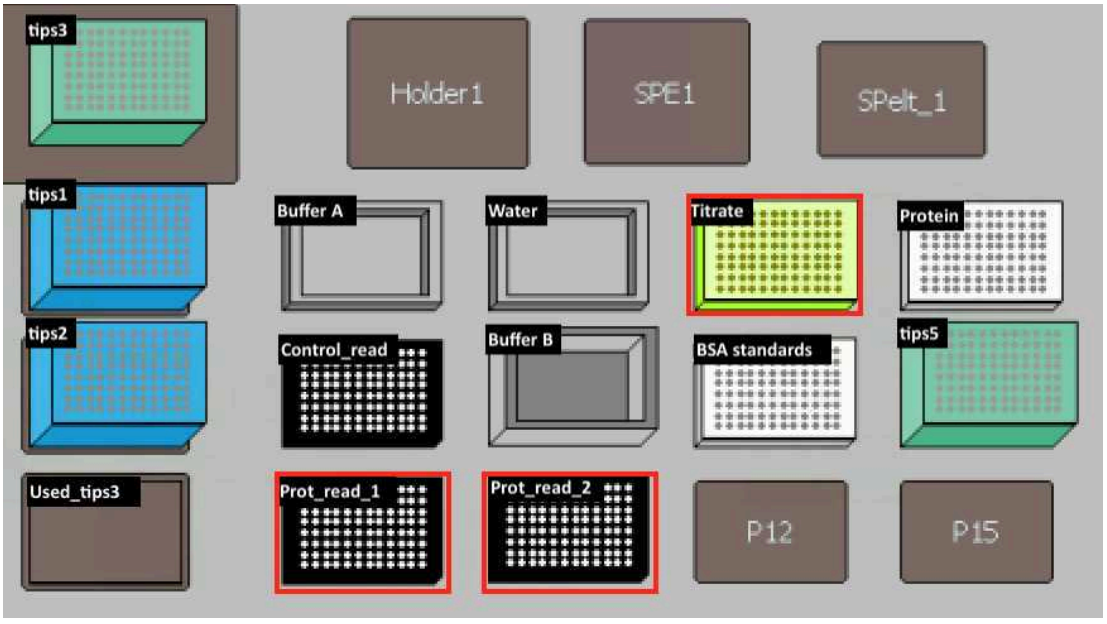
1m

Note

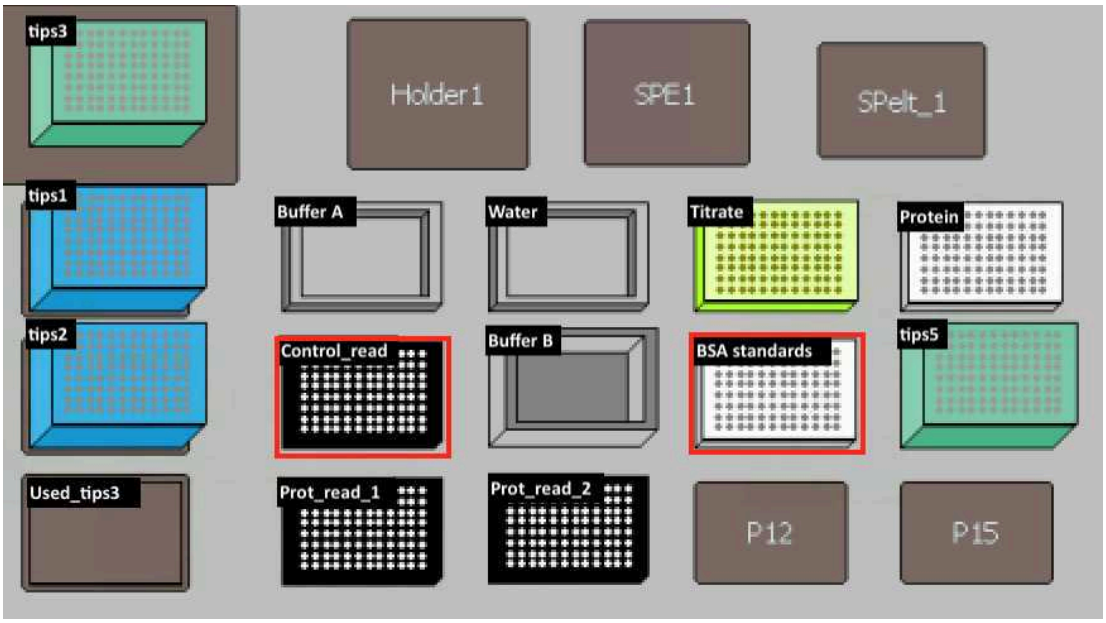
Note: The dilution factor could be altered as needed by changing the volumes of water and proteins transferred to the titration plate. Be sure to multiply the protein quant results by the dilution factor before you do your normalization calculation.



8 Transfer 5 µl of protein from Titrate plate to Protein Read plate 1 and Protein Read plate 2. 1m



9 Transfer 5 µl from BSA Standards plate to Control Read plate. 1m



Note

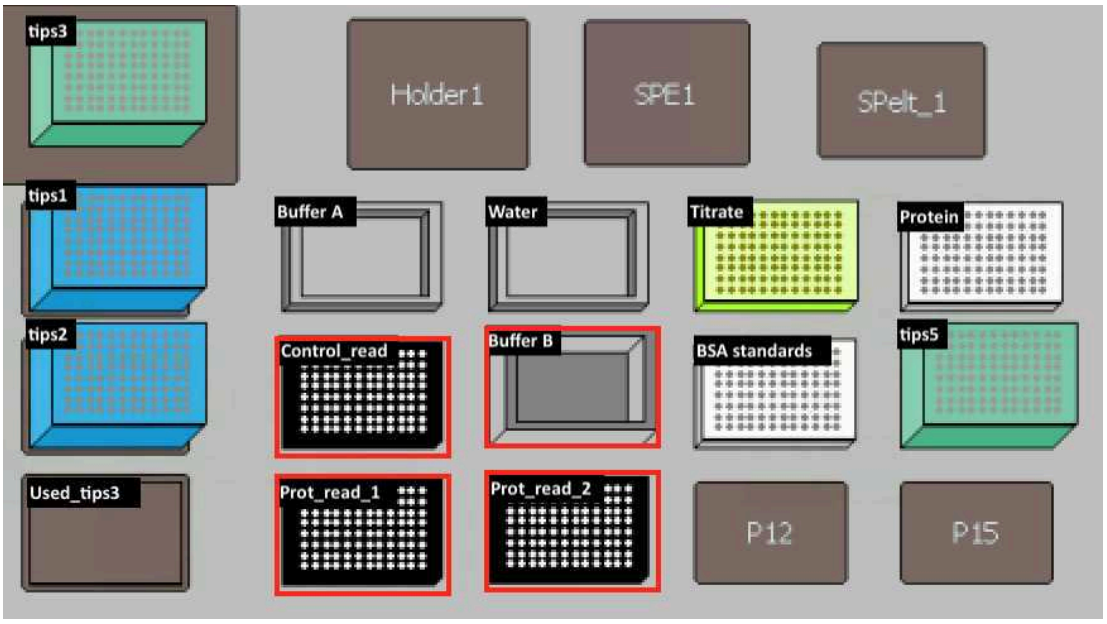
Prepare BSA Standards plate (1st 4 rows from A to D):
Add 40 µl of H_2O into wells A1 to D1.
Add 40 µl of BSA Standards 1 (125 µg/ml) to 7 (2000 µg/ml) into columns 2 to 8.

10 Method will pause until user resumes it again. Set a timer for 5 minutes. 5m

11 After 5 min, click OK to resume method.

12 Transfer 200 µl from Buffer B to the 3 Read plates and incubate for 00:10:00 . 10m

The method will pause until user resumes it again. Set up a 10 minutes timer and click OK afterwards to finish.



Spectrophotometer reading for protein Quantification

10m

13 Transfer plates to the microplate reader (MD Spectramax 250) to read absorbance at 750 nm and calculate protein concentration.

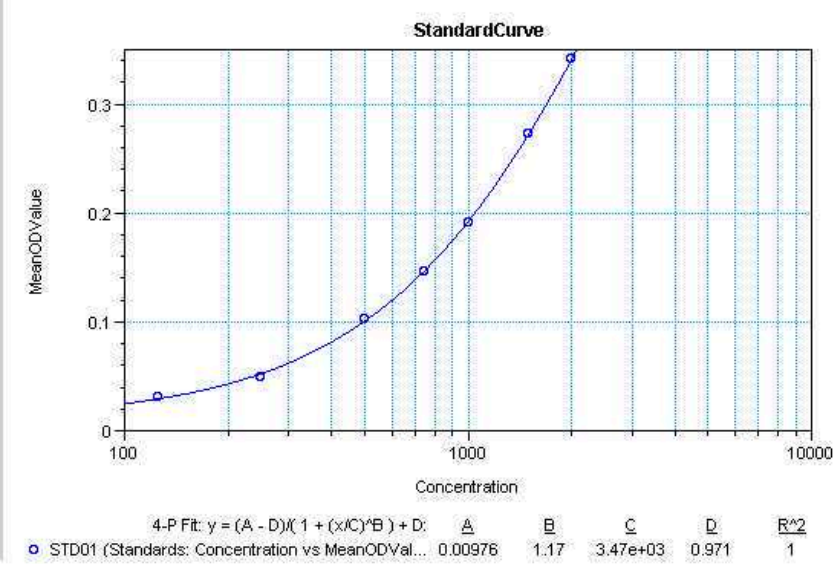
1m

14 Read Control Read plate.

1m

	A	B	C
	Sample	**Concentration**	**Mean OD Value (Absorbance)**
	St01	125	0.024
	St02	250	0.042
	St03	500	0.090
	St04	750	0.138
	St05	1000	0.165
	St06	1500	0.239
	St07	2000	0.281

Standards (µg/ml)



Example Standards Curve

15 Read Protein Read plate 1 and Protein Read plate 2. 2m

	A	B
	Sample	**Concentration**
	Un88	585.249
	Un89	785.257
	Un90	670.135
	Un91	718.864
	Un92	868.962
	Un93	679.907
	Un94	743.064
	Un95	994.173

	A	B
	Un96	1115.072

Examples of Sample concentrations that are reported by MD Spectramax 250

Note

Remember to multiply the protein concentrations by five (5) to account for the five-fold dilution in Step #7.

16 Store protein plate at -20 °C until ready for **Automated Protein Normalization and Tryptic Digestion on a Biomek-NX Liquid Handler System**.