User Guide

Protein (P2) Cartridge Kit (C105121/C105221)

A. Specifications

Specification	Description
Protein Sizing Range	11-155 kDa
L.O.D	$Qsep_1$/$Qsep_1$-**Plus**/$Qsep_{100}$: 5 ng/µL (Chromeo-BSA) $Qsep_{100}$ **Advance**: 0.5 ng/µL (Alexa-BSA)
Sample Number	100 runs
Shelf Life	4 months

B. Kit Components and Storage Conditions

Item	Storage Condition
Protein Cartridge (C105121/C105221)	15-27°C (Do Not Freeze)
5X Separation Buffer (SDS) (C104504-5X, 50 mL)	15-27°C
Protein Dilution Buffer (C104505, 15mL)	15-27°C

C. Additional Kits for Purchase

		Model	
		$Qsep_1$/$Qsep_1$-**Plus**/ $Qsep_{100}$	$Qsep_{100}$ **Advance**
Labeling Kit	**Item**	Protein Labeling Kit (Chromeo P503)	Protein Labeling Kit (Alexa Fluor 488)
	Manufactory	BiOptic Inc.	BiOptic Inc.
	Cat. No.	C104600	C104800
Labeling Dye	**Item**	Chromeo™ P503 (1mg)	Alexa Fluor™ 488 NHS Ester (Succinimidyl Ester)
	Manufactory	Sigma-Aldrich	ThermoFisher Scientific
	Cat. No.	30693	A20000 & A20100
Protein Standard	**Item**		BenchMark™ Fluorescent Protein Standard (125 µL)
	Manufactory		ThermoFisher Scientific
	Cat. No		LC5928

D. Protocol Steps

The table below provides an overview of the protein labeling workflow.

Step	Page
Reagent Preparation	
Sample Preparation	$Qsep_1$/$Qsep_1$-**Plus**/$Qsep_{100}$: 3
Protein Labeling	$Qsep_{100}$ **Advance:** 4-5
Labeled Protein Treatment	*Additional kits may need to be purchased.
Sample Analysis	

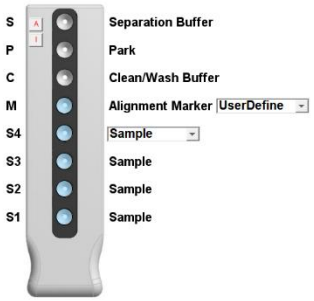
E. Buffer Preparation for Cartridge Unpacking

New cartridge must perform HV purge before use. Please fill 1X Separation Buffer into S, P, W and C wells. Each well should only be filled to the groove of wells. Then, select "Direct Control" on the control panel and click "Go" to start HV purge. The condition should be set to 4kV, 120s.

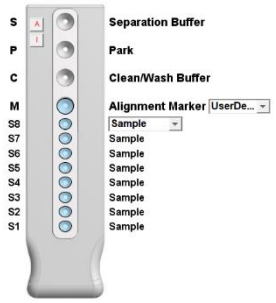
- Separation Buffer (1X): 5X dilution from the stock with ddH_2O.

$Qsep_{100}$

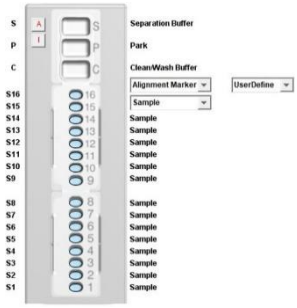
$Qsep_1$ 8-well tray



$Qsep_1$ 12-well tray



$Qsep_1$-Plus
16+3 well tray



F. Sample Preparation

Sample Volume Requirements

0.2 mL Tube: 20 µL

0.1 mL Tube: 10 µL

Micro Vial (C104250): 2 µL

Recommended Sample Concentration

Final Protein Sample: 50-100 ng/µL

***NOTE: Use Protein Dilution Buffer (C104505) to dilute labeled protein samples to proper concentrations. For $Qsep_1$/$Qsep_1$-Plus/$Qsep_{100}$, dilute protein samples 20X-50X and for $Qsep_{100}$ Advance, dilute protein samples 200X-500X.**

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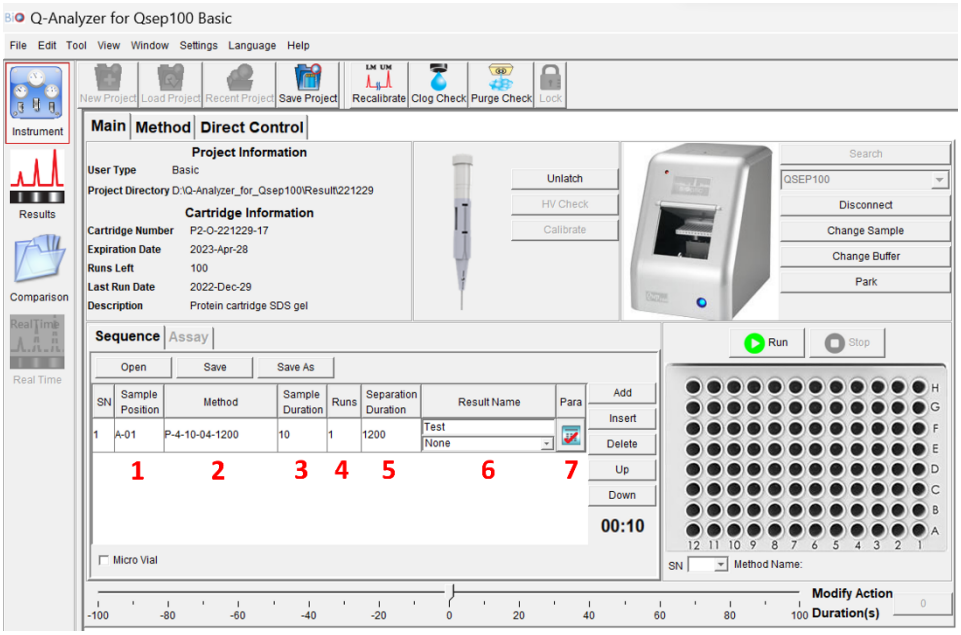
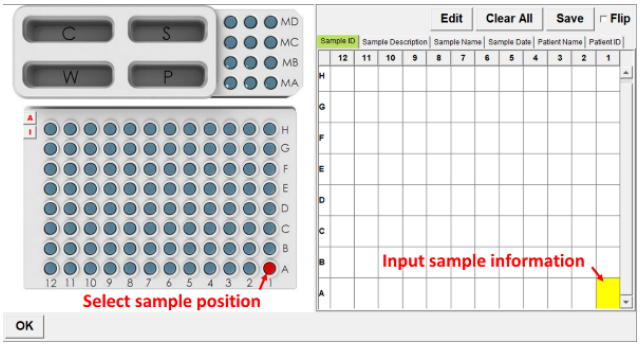
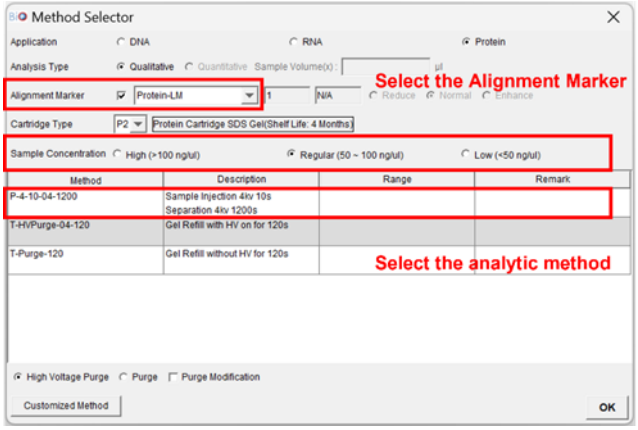
G. Software Operation



1. Place the sample and select corresponding positions, and then input sample information if necessary.



2. Select the alignment marker (Protein-LM or User Define) and the analytic method in Method Selector.



*NOTE: Based on sample concentration to adjust injection condition.

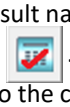
Sample Concentration	High (2kV, 10s)	Regular (4kV, 10s)	Low (8kV, 10s)
Protein	> 100 ng/μL	1-100 ng/μL	< 1 ng/μL

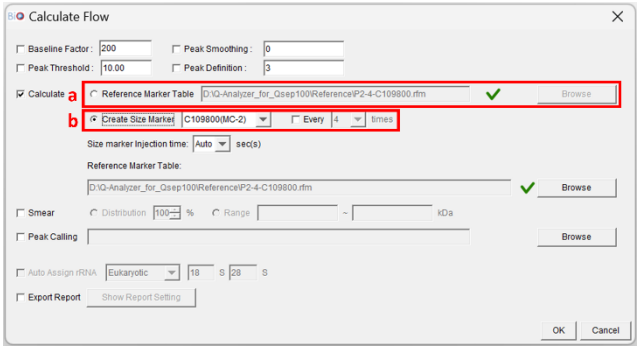
3. Sample Duration: adjust the sample injection time to increase/decrease injection amount.

   *NOTE: Do not set the injection time over 10 sec.

4. Runs: repetition time.
5. Separation Duration: adjust the duration to extend/reduce the separation time.

   *NOTE: Step 3-5 are optional.

6. Input the result name for the result file.
7. Click "Para"  . Choose (a) reference or (b) create size marker to do the calculations.



*NOTE: When using function "Create Size Marker", select C109800 to pair with the Lower Marker.

8. Click "Run"  to start the analysis.

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Protein Labeling Kit (C104600) for $Qsep_1$/$Qsep_1$-Plus/$Qsep_{100}$

A) Kit Components and Storage Conditions

Item	Storage Condition
5X Labeling Buffer (C104601, 8 mL)	4°C
Denaturant (C104602, 500 µL)	15-27°C
Protein Alignment Marker (C104605, 100 µL x2)	15-27°C

Protein Labeling Dye is NOT included in Protein Labeling Kit.

Order information of the Protein Labeling Dye:

Item	Manufactory / Cat. No.	Storage Condition
Chromeo™ P503 (1mg) (C104600)	Sigma-Aldrich: 30693	Short-Term: 4°C Long-Term: -20°C (avoid the light)

Protein labeling dye needs to be dissolved in proper solvents Dimethylsulfoxide (DMSO), Dimethylformamide (DMF), Acetonitrile or Methanol (DMSO is recommended).

B) Reagent Preparation

- Labeling Buffer (1X): 5X dilution from the stock with ddH2O.
- Dye Stock Solution: Dissolve the Protein Labeling Dye in 1mL DMSO (1 mg/mL) and cover the dye with aluminum foil to avoid the light (stored at -20°C).
- Dye Working Solution: 5X dilution from the Dye Stock Solution with DMSO (0.2 mg/mL before labeling).

C) Sample Preparation

Dissolve the protein sample with 1X Labeling Buffer and follow the instructions below. (Before labeling, the concentration of protein is 2 mg/mL).

D) Protein Labeling Protocol

Reagent	Volume (µL)
1X Labeling Buffer	12
Protein Sample (2 mg/mL)	5
Denaturant (C104602)	2
Dye Working Solution	1
Total Volume	**20**

1. Add the reagents as table above into 0.2 mL PCR tubes and mix gently.
2. Label the protein samples at 60°C for 10 minutes (covered with aluminum foil to avoid the light).
3. Cool down the samples to room temperature.
4. Store the protein samples at -20°C.

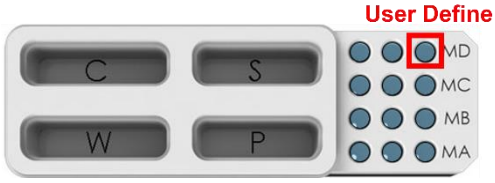
***NOTE: Protein properties, including pI and structure, could affect the labeling efficiency.**

E) Labeled Protein Treatment

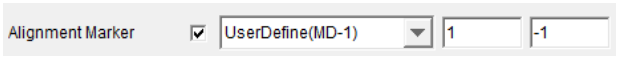
1. Use Protein Dilution Buffer (C104505) to dilute labeled protein samples to proper concentrations (50-100 ng/µL). Diluting protein samples 20X-50X is recommended.
2. Before analyzing, protein samples need to be heated at 100°C for 5 minutes. Once the samples cool down to room temperature, follow the instructions to analyze the samples.

F) Sample Analysis

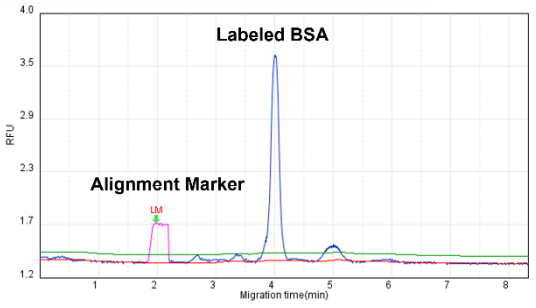
1. Place the samples into the instrument.
2. Place the Protein Alignment Marker (C104605) at MD-1.



3. Set the Alignment Marker to User Define and enter 1 and -1 for lower and upper markers, respectively.



G) Result



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Protein Labeling Kit (C104800) for *Qsep*$_{100}$ Advance

A) Kit Components and Storage Conditions

Item	Storage Condition
5X Labeling Buffer (C104601, 8 mL)	4°C

Protein Labeling Dye and Protein Standard are NOT included in Protein Labeling Kit.

Order information of the Protein Labeling Dye:

Item	Manufactory / Cat. No.	Storage Condition
Alexa Fluor™ 488 NHS Ester (Succinimidyl Ester) (C104800)	ThermoFisher Scientific: A20000 (1 mg) A20100 (5 mg)	≤ -20°C (avoid the light)

Protein labeling dye needs to be dissolved in proper solvents: High-quality, anhydrous dimethylformamide (DMF) or dimethylsulfoxide (DMSO) (DMSO is recommended).

Order information of the Protein Standard:

Item	Manufactory / Cat. No.	Storage Condition
BenchMark™ Fluorescent Protein Standard (125 µL)	ThermoFisher Scientific: LC5928	-30°C to -10 °C (avoid the light)

The Protein Standard consists of fluorescent dye-conjugated proteins, ranging in size from 11kDa to 155kDa.

B) Reagent Preparation

- Labeling Buffer (1X): 5X dilution from the stock with ddH2O.
- Dye Stock Solution: Dissolve the Protein Labeling Dye (5mg) in 500 µL DMSO (10 mg/mL), aliquot, and cover the dye with aluminum foil to avoid the light (stored at -20°C).
- Dye Working Solution: 10X dilution from the Dye Stock Solution with DMSO (1 mg/mL before labeling).

C) Sample Preparation

Dissolve the protein sample with 1X Labeling Buffer and follow the instructions below. (Before labeling, the concentration of protein is 2-10 mg/mL. Concentration lower than 2 mg/mL will greatly decrease the efficiency of the reaction.)

D) Protein Labeling Protocol

Reagent	Volume (µL)
Protein Sample (2-10 mg/mL)	18
Dye Working Solution	2
Total Volume	**20**

1. Add the reagents as table above into 0.2 mL PCR tubes and mix gently.
2. Incubate the reaction for 1 hour at room temperature (covered with aluminum foil to avoid the light).
3. Store the protein samples at -20°C.

***NOTE: The recommended molar ratio between Dye and Protein Sample is 2:1 to 10:1.**

***NOTE: Protein properties, including pI and structure, could affect the labeling efficiency.**

E) Labeled Protein Treatment

1. Use Protein Dilution Buffer (C104505) to dilute labeled protein samples to proper concentrations (50-100 ng/µL). Diluting protein samples 200X-500X is recommended.
2. Before analyzing, protein samples need to be heated at 100°C for 5 minutes. Once the samples cool down to room temperature, follow the instructions to analyze the samples.

F) Protein Standard Treatment and Preparation

1. Thaw the BenchMark™ Fluorescent Protein Standard at room temperature.
2. Dilute the Protein Standards 10X with Protein Dilution Buffer (C104505), which is included in the Protein Cartridge Kit.
3. Before analyzing, the protein standards need to be heated at 100°C for 5 minutes. Once the samples cool down to room temperature, follow the instructions to analyze the samples.

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Protein Labeling Kit (C104800) for *Qsep*$_{100}$ Advance

Use Alexa Fluor™ 488 dye as lower marker to align labeled proteins.

1. Dilute the Dye Stock Solution (10 mg/mL) 200X with DMSO.
2. Dilute the Dye Solution from step 1 100X with 1X Labeling Buffer.
3. Add the reagents as followings into 0.2 mL PCR tubes.

Reagent	Volume (µL)
BenchMark™ Fluorescent Protein Standard	3
Dye Solution from Step 2	1
Protein Dilution Buffer (C104505)	26
Total Volume	**30**

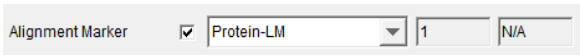
   I. Add 3 µL BenchMark™ Fluorescent Protein Standard into 0.2 mL PCR tube.
   II. Add 1 µL Dye Solution from step 2.
   III. Add 26 µL Protein Dilution Buffer.

4. Before analyzing, protein standards need to be heated at 100°C for 5 minutes. Once the samples cool down to room temperature, follow the instructions to analyze the samples.

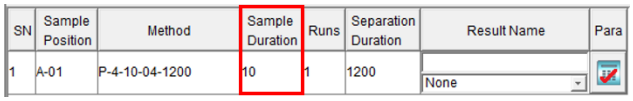
G) Sample Analysis

1. Place the samples into the instrument.
2. Place the Protein Standards at SM02, SM05, SM08, and SM11 to create Size Marker.

3. Set the Alignment Marker to Protein-LM (the lower marker must be mixed with the Protein Standard).



4. Adjust the sample injection duration accordingly.



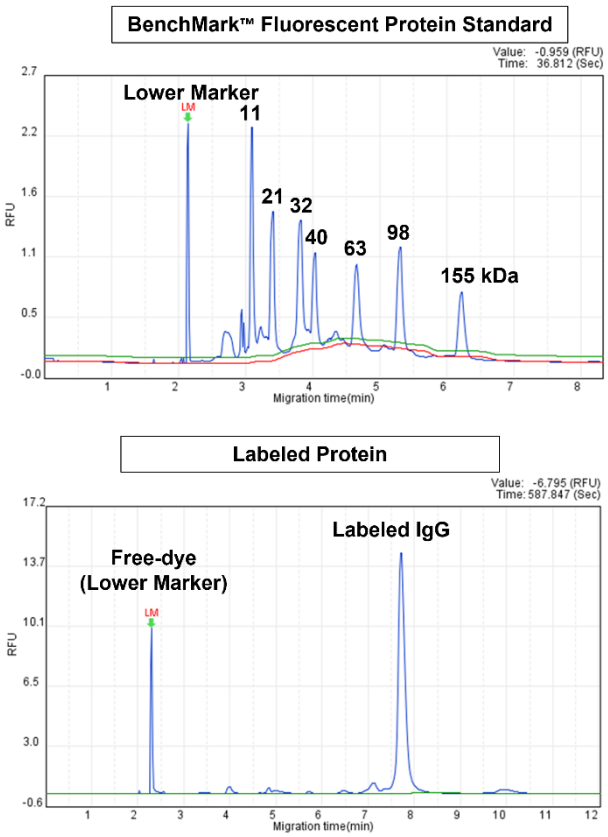
SN	Sample Position	Method	Sample Duration	Runs	Separation Duration	Result Name	Para
1	A-01	P-4-10-04-1200	10	1	1200	None	

Recommended Sample Injection Duration:

For Protein Standard: 10s

For Labeled Protein: 1-5s

H) Result



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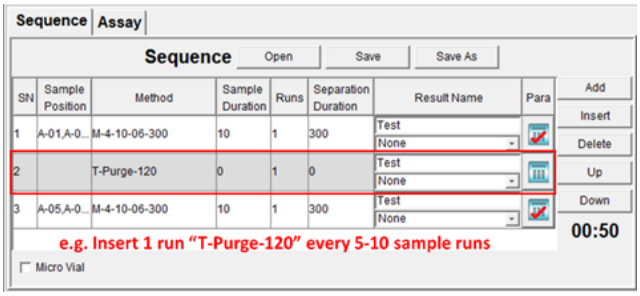
H. Troubleshooting

Chromeo™ P503 and Alexa Fluor™ 488 here are used as a fluorogenic reagent to label primary amine groups (R-NH_2) within proteins molecules. Using amine-containing solutions or buffers, such as Tris, as a solvent should be avoided to prevent them from competing for conjugation with amine-reactive compounds.

Please ensure the whole system is working well and the operation is following the instructions.

Sometimes, unknown substances may cause unstable current in sample injection or separation steps. Here is a list of solutions to help fix the occurrence.

1. Use dilution buffer to dilute the sample.
2. Centrifuge the sample for a while to make the residues accumulate at the bottom of the tube.
3. Insert a “T-Purge-120” method between sample runs.
   E.g., Insert a “T-Purge-120” every 5-10 sample runs.



I. Cartridge Discard

Please wear gloves before discarding the cartridge.

1. Bend the cartridge tips.
2. Open the caps on gel reservoir and remove the inner caps.
3. Pour the gel into the chemical waste container.
4. Cartridge can be thrown into the bin.

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