User Guide
Protein (P2) Cartridge Kit (C105121/ C105221) on *Qsep*$_1$/ *Qsep*$_{100}$

A. Specifications

Specification	Description
Protein Sizing Range	11-155 kDa
L.O.D	5 ng/μL
Sample Number	100 runs
Shelf Life	4 months

* LOD is determined by BSA labeled by Chromeo P503.

B. Kit Components and Storage Conditions

- Protein Cartridge Kit (C105121/ C105221)

Item	Storage Condition
Protein Cartridge (C105121/C105221)	2-8°C (Do Not Freeze)
5X Separation Buffer (SDS) (C104501-5X, 50 mL)	15-27°C
Protein Dilution Buffer (C104505, 15 mL)	15-27°C

⚠ **Please always store cartridges in a light-proof bag and then store in the cartridge box after analysis.**

⚠ **Warm to room temperature for 30 min before cartridge use.**

- Protein Labeling Kit (C104600)

Item	Storage Condition
5X Protein Labeling Buffer (C104601-5X, 8 mL)	4°C
Denaturant (C104602, 500 μL)	15-27°C
Protein Alignment Marker (C104605, 100 μL*2 pcs)	15-27°C

C. Additional Kits for Purchase

Item	Manufacturer/ Cat. No.	Storage Condition
Protein Labeling Kit (Chromeo P503)	BiOptic Inc./ C104600	Store components as instructed.
Chromeo™ P503	Sigma-Aldrich/ 30693 (1 mg)	≦ -20°C (avoid the light)

D. Protocol Steps

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

Step1: Reagents preparation
↓
Step2: Sample preparation
↓
Step3: Labeled Protein Treatment
↓
Step4: Sample Analysis

E. Reagents Preparation

E-1. Separation buffer:

☐ 5X Stock: supplied by Protein Cartridge kit (C105121/ C105221)

- **1X working separation buffer preparation:**
  5X dilution from the 5X separation buffer stock with ddH_2O.

E-2. Protein Dilution buffer:

- The Dilution Buffer provided in the cartridge kit (C105121 / C105221) is **ready-to-use**. Do not dilute the Dilution Buffer before use.

E-3. Labeling buffer:

☐ 5X Stock (C104601-5X): supplied by Protein Labeling kit (C104600)

- **1X working labeling buffer preparation:**
  5X dilution from the 5X labeling buffer stock with ddH_2O.

E-4. Dye solution:

- **Dye Stock Solution (1 mg/mL) preparation:**
  Dissolve the Protein Labeling Dye (1mg) in 1 mL DMSO.
- **Dye working solution (0.2 mg/mL) preparation:**
  5X dilution from the Dye stock solution with DMSO.

* Aliquot and cover the dye with aluminum foil to avoid the light.

* Prepare Dye working solution before labeling procedure.

F. Sample Preparation

The diagram below provides an overview of the protein sample preparation.

Dissolve or dilute protein samples
↓
Mix protein sample and dye
↓
Incubate sample and dye mixture
↓
Dilute sample mixture to proper concentration
↓
Denature sample mixture before analysis

Buffer Requirements for Protein Sample:

Category	Specification
Recommended Buffers	**• 1X PBS (Without Phenol Red)** **• Use 50–100 mM of one of the following:** **- Sodium bicarbonate** **- Sodium phosphate** **- Sodium borate**
Required pH Range	**pH 7.0 – 9.0**
Prohibited Components	**- Buffers containing amine groups (e.g., Tris)** **- Detergents (e.g., SDS)** **- Reducing agent (e.g., DTT, β-ME)**

User Guide
Protein (P2) Cartridge Kit (C105121/ C105221) on $Qsep_1$/ $Qsep_{100}$

Step 1: Dilute or dissolve the protein samples to a final concentration of 2–10 mg/mL using 1X Labeling Buffer (E-3). Mix gently to ensure complete dissolution before proceeding to the next step.

Step 2: Mix the reagents as followings into 0.2 mL PCR tubes as protein sample mixture.

Reagent	Volume (µL)
Protein Sample (2-10 mg/mL)	5
1X Labeling Buffer (E-3)	12
Denaturant (C104602)	2
1x Dye Working Solution (E-4)	1
Total Volume	20

*Note: Sample concentrations below 2 mg/mL may significantly reduce the efficiency of the reaction.

Step 3: Incubate sample mixture 60°C for 10 mins, covering the tube with aluminum foil to protect the sample from light.

*Note: If immediate analysis is not planned, store the sample mixture at –20 °C until use.

Step 4: Cool the samples to room temperature before proceeding to the next step.

Step 5: Dilute the protein sample to a final concentration of 10–50 ng/µL using Dilution Buffer (E-2) after incubation.

*Note: For example, a 2 mg/mL sample should be diluted 20X–50X with Dilution Buffer (E-2) to reach a final concentration of 10–50 ng/µl.

Step 6: Heated sample mixture at 100°C for 5 mins.

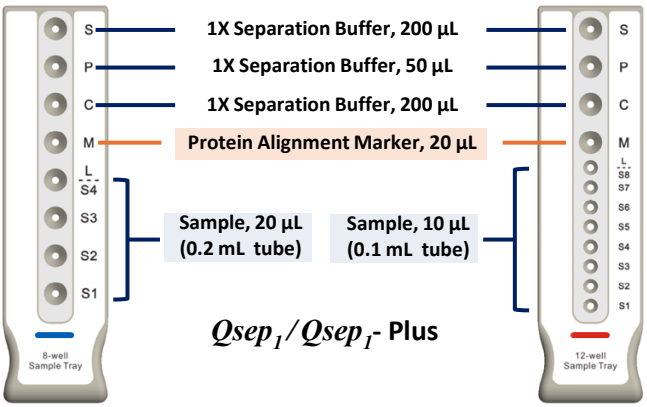
*Note: Protein properties, including pI and structure, could affect the labeling efficiency.

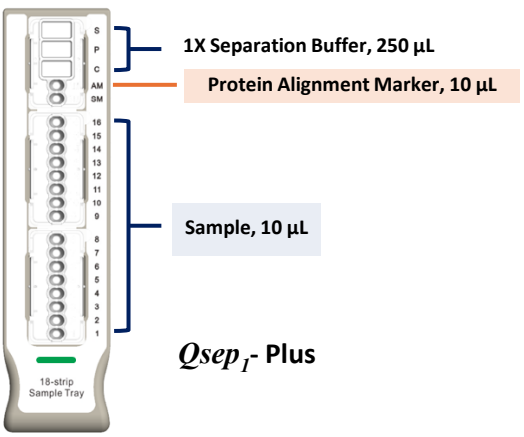
G. Cartridge Preparation

New cartridge must undergo “Purge and Verify” before use. Please follow the instructions provided in the unpacking guide.

H. Buffer and Marker Preparation

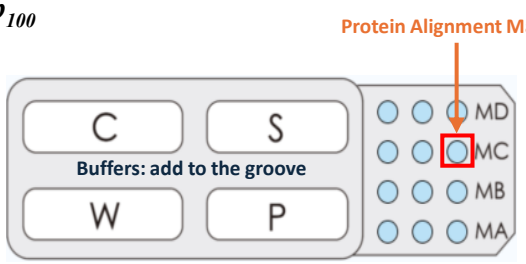
- 1X Separation Buffer (E-1): 5X dilution from the 5X separation buffer with ddH2O.
- Fill 1X Separation buffer into S, P, W and C wells.
- Place a sufficient volume of the Protein Alignment Marker in the designated position, according to the type of tray used.





- **Ensure the buffer tray is pushed to the end until the color bar aligns with the edge of the holder.**

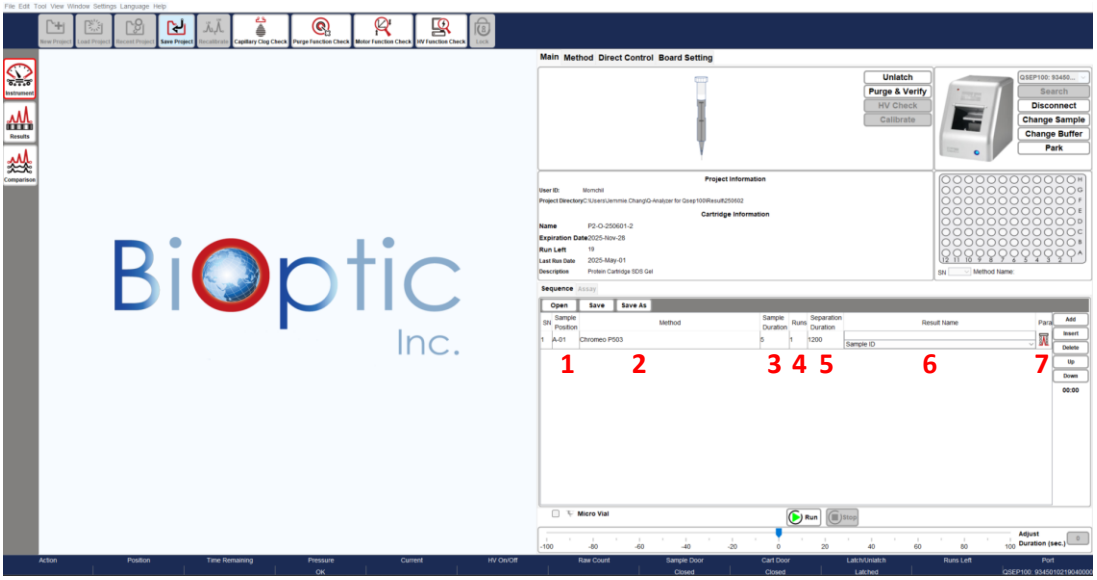
$Qsep_{100}$



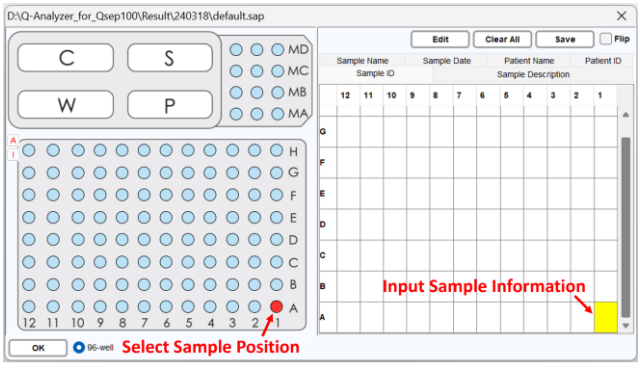
P/W/C/S: 1X Separation Buffer
MC1: Protein Alignment Marker

User Guide
Protein (P2) Cartridge Kit (C105121/ C105221) on $Qsep_1$/ $Qsep_{100}$

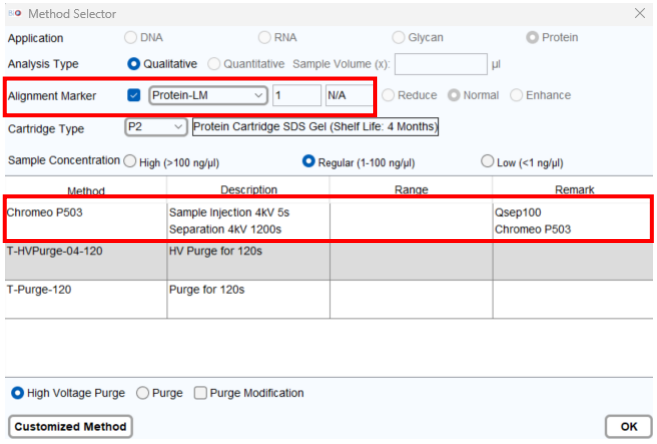
I. Software Operation Guide



1. Sample Position: Place the sample and select the corresponding position. Input sample information if necessary.



2. Method: Select (a) Alignment Marker and (b) Analytic Method in the Method Selector.



- **Adjust injection conditions based on sample concentration.**

Sample Concentration	High (2kV, 5s)	Regular (4kV, 5s)	Regular (8kV, 5s)
Protein	>100 ng/μL	1-100 ng/μL	<1 ng/μL

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

- **Modify injection conditions based on sample concentration.**

4. Runs: Set the repetition time.

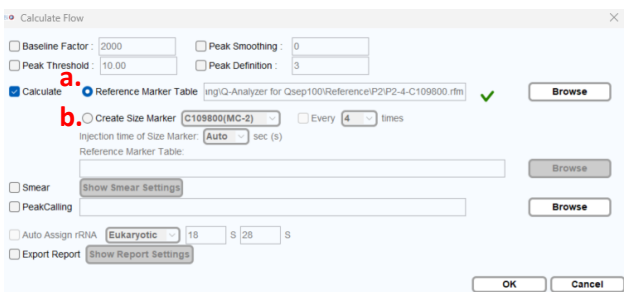
5. Separation Duration: Adjust the duration to extend/reduce the separation time.

(Optional)

6. Result Name: Input the result name for the result file.

- Para: Choose between (a) Reference Marker Table and (b) Create Size Marker for calculation.

- Note: Activation of the "Create Size Marker" function is contingent upon the presence of a protein ladder labeled Chromeo-P503. In the absence of the appropriate ladder, users are advised to select the "Reference Marker Table".



- When using function "Create Size Marker" function, ensure protein size marker which labeled Chromoe-P503 is placed at MC2.

8. Click "Run" to start the sequence analysis.

User Guide
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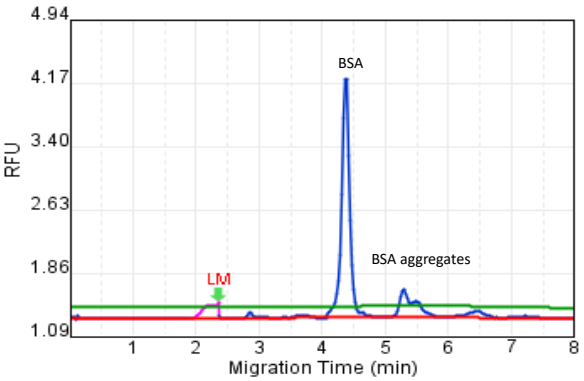
J. Results

Labeled BSA



K. Troubleshooting

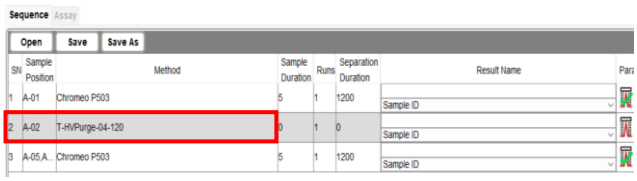
Chromeo™ P503 here are used as a fluorogenic reagent to label primary amine groups (R-NH_2) within proteins molecules. Using amine-containing solutions or buffers, such as Tris, as a solvent should be avoided to prevent them from competing for conjugation with amine-reactive compounds.

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.



L. Cartridge Disposal

Please wear gloves before discarding the cartridge.

Gel Reservoir

1. Bend the cartridge tip.
2. Open the cap on the gel reservoir and remove the inner cap.
3. Pour the gel into the chemical waste container.
4. Dispose of the cartridge in the trash bin.

Contact Information:
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