

User Guide

Protein (P2) Cartridge Kit (C105121/ C105221) on *Qsep₁₀₀* Advance

A. Specifications

Specification	Description
Protein Sizing Range	11-155 kDa
L.O.D	0.5 ng/μL
Sample Number	100 runs
Shelf Life	4 months

* LOD is determined by BSA labeled by Alexa488.

B. Kit Components and Storage Conditions

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.

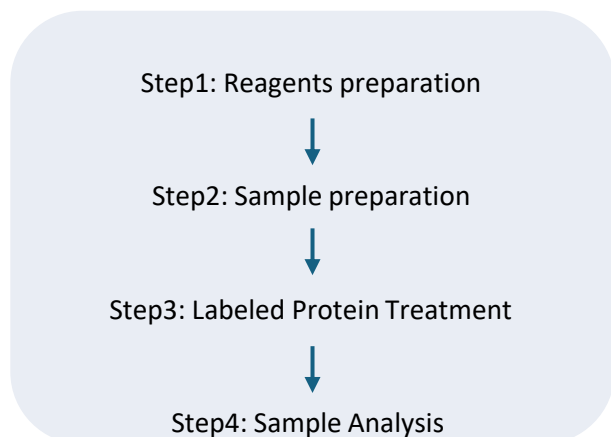
⚠ Warm to room temperature for 30 min before cartridge use.

C. Additional Kits for Purchase

Item	Manufacturer/ Cat. No.	Storage Condition
Protein Labeling Kit (Alexa Fluor 488)	BiOptic Inc./ C104800	4°C
Alexa Fluor™ 488 NHS Ester (Succinimidyl Ester)	ThermoFisher Scientific/ A20000 (1 mg) A20100 (5 mg)	≤ -20°C (avoid the light)
BenchMark™ Fluorescent Protein Standard (125 μL)	ThermoFisher Scientific/ LC5928	-30°C to -10°C (avoid the light)

D. Protocol Steps

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



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 ddH₂O**.

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 (C104800)
- **1X working labeling buffer preparation:**
5X dilution from the **5X labeling buffer stock with ddH₂O**.

E-4. Dye solution:

- **Dye Stock Solution (10 mg/mL) preparation:**
Dissolve the Protein Labeling Dye (5mg) in 500 μL DMSO.
- **Dye working solution (1 mg/mL) preparation:**
10X dilution from the **Dye stock solution with DMSO**.

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

E-5. Protein Standard (Optional):

Step 1: Dilute Dye stock solution (10 mg/mL) 200 times with **DMSO** as intermediate dye solution.

Step 2: Dilute intermediate dye solution (E-5, Step 1) 100 times with **1x labeling buffer (0.5 ng/mL)** as dye working solution for protein standard.

*Please make sure to mix thoroughly after each dilution step.

Step 3: Mix the reagents as followings into 0.2 mL PCR tubes as protein standard mixture

Reagent	Volume (μL)
BenchMark™ Fluorescent Protein Standard	3
Dye Working Solution for protein standard (E-5, Step 2)	1
Protein Dilution buffer (E-2)	26
Total Volume	30

Step 4: Heated protein standard mixture at 100°C for 5 mins.

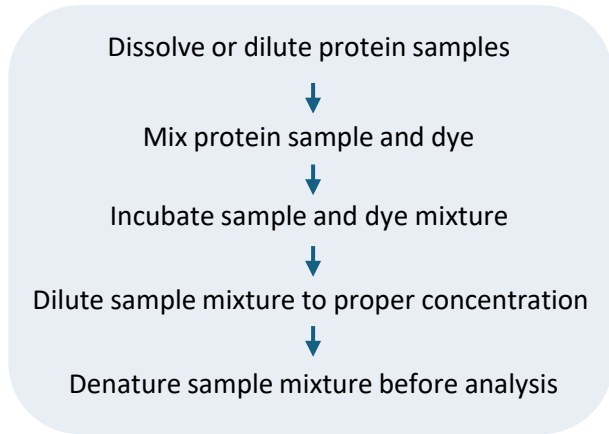
*The intermediate dye solution is suitable for long-term use when protected from light with aluminum foil and properly stored at -20 °C.

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F. Sample Preparation

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



Buffer Requirements for Protein Sample:

Category	Specification
Recommended Buffers	<ul style="list-style-type: none"> • 1X PBS (Without Phenol red) • Use 50–100 mM of one of the following: <ul style="list-style-type: none"> - Sodium bicarbonate - Sodium phosphate - Sodium borate
Required pH Range	pH 7.0 – 9.0
Prohibited Components	<ul style="list-style-type: none"> - Buffers containing amine groups (e.g., Tris) - Detergents (e.g., SDS) - Reducing agent (e.g., DTT, β-ME)

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)	18
Dye Working Solution (E-4)	2
Total Volume	20

⚠ Sample concentrations below 2 mg/mL may significantly reduce the efficiency of the reaction.

Step 3: Incubate sample mixture at room temperature for 1 hour, 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.

*Note: If precipitation or turbidity is observed during sample preparation, it may lead to reduced labeling efficiency. Users are advised to check the protein characteristics, including the isoelectric point (pI) and the composition of the dissolution buffer.

Step 4: 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 40X–200X with Dilution Buffer (E-2) to reach a final concentration of 10–50 ng/μL.

Step 5: Heated sample mixture at 100°C for 5 mins. Then cool the samples to room temperature before proceeding to the next step.

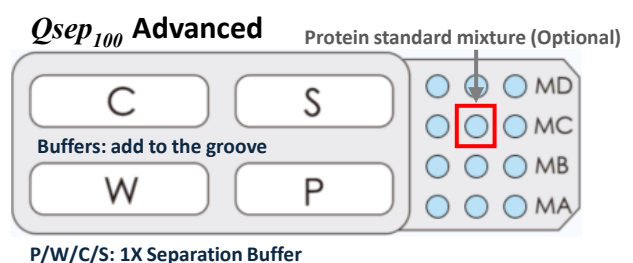
*Note: Protein properties, including pI and structure, could affect the labeling efficiency.

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

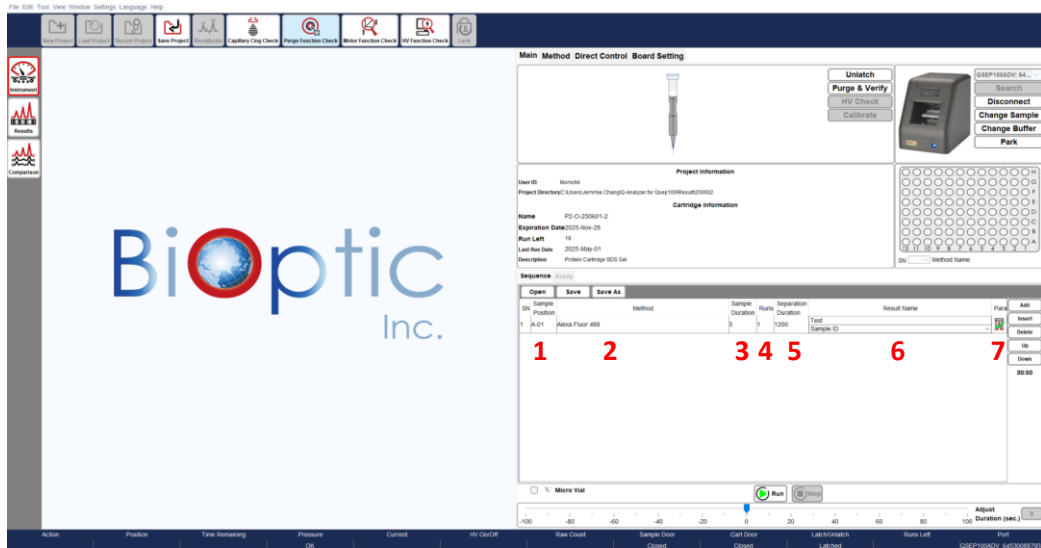
- 1X Separation Buffer: 5X dilution from the 5X separation buffer with ddH₂O.
- Please fill 1X Separation buffer into S, P, W and C wells. Each well should only be filled to the groove of wells.
- Place protein standard mixture at MC2 position. (Optional)



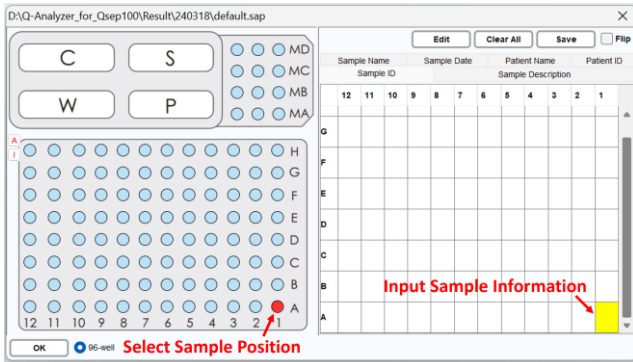
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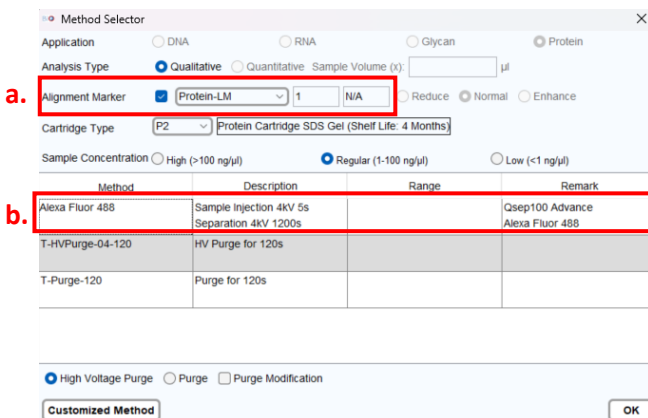
I. Software Operation Guide



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.

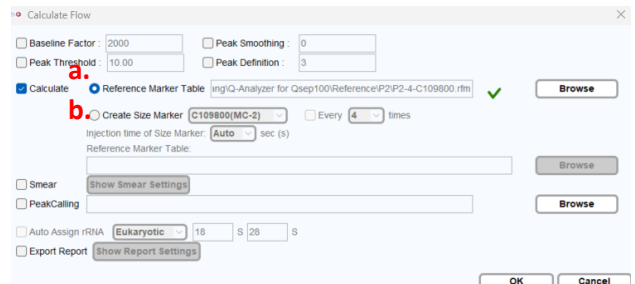
4. Runs: Set the repetition time.

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

(Optional)

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

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



• When using function "Create Size Marker" function, ensure protein standard mixture is placed at MC2.

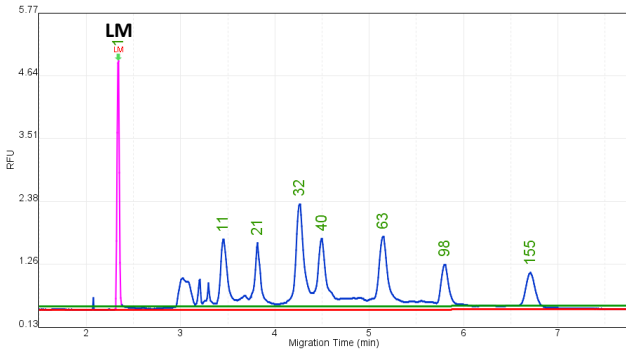
8. Click "Run" to start the sequence analysis.

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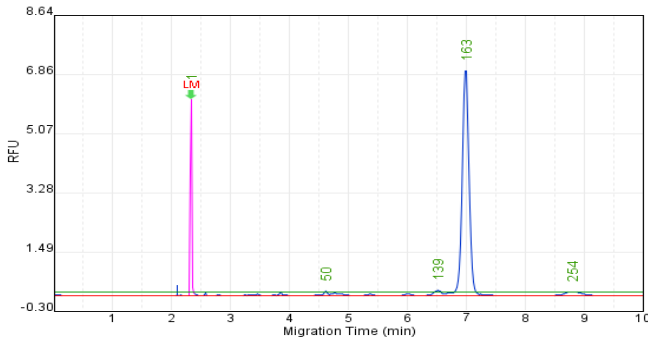
Protein (P2) Cartridge Kit (C105121/ C105221) on Qsep100 Advance

J. Results

BenchMark™ Fluorescent Protein Standard



Labeled IgG



K. Troubleshooting

Alexa Fluor™ 488 here are used as a fluorogenic reagent to label primary amine groups (R-NH₂) 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.

Sample Position	Method	Sample Duration	Runs	Separation Duration	Result Name
1 A-01	Alexa Fluor 488	5	1	1200	Test Sample ID
2	T-Purge-04-120	0	1	0	Test Sample ID
3 A-03	Alexa Fluor 488	5	1	1200	Test Sample ID

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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