User Guide of Protein Labeling Kit (Alexa Fluor™ 488) (Cat. No.C104800)

Notes before starting:

- **The properties of the proteins including, pI and structure could affect the labeling efficiency.**
- **Protein labeling Dye and Protein Standard are NOT included in the labeling kit.**

Order information of the Protein labeling Dye:

Product Description	Manufactory / Cat. No.	Storage condition
Alexa Fluor™ 488 NHS Ester (Succinimidyl Ester)	ThermoFisher Scientific: A20000 (1 mg) A20100 (5 mg)	≦-20℃(Avoid the light)

** Protein labeling dye needs to be dissolved in proper solvents as below.

High-quality, anhydrous dimethylformamide (DMF) or dimethylsulfoxide (DMSO) **(Recommend: DMSO)**

Order information of the Protein Standard:

Product Description	Manufactory / Cat. No.	Storage condition
BenchMark™ Fluorescent Protein Standard (125 µl)	ThermoFisher Scientific: LC5928	-30℃ to -10℃(Avoid the light)

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

Contents:

Packing List: (200 Reactions)

Cat. No.	Description	Size	Storage condition
C104601	5X Labeling Buffer	8 ml	4℃

Instruction of Protein Labeling process:

Preparation of reagents：

1X Labeling Buffer: Use 1:4(v/v) ratio to dilute **5X Labeling Buffer** by Deionized water.

Dye Stock Solution: To dissolve the **Protein labeling Dye (5 mg)** by **500 µl** DMSO**(10 mg/ml)**, aliquot, and cover by the aluminum foil to avoid the light (Store at -20℃)

Dye Working Solution: Use 1:9(v/v) ratio to dilute the **Dye Stock Solution** by DMSO, before labeling **(1 mg/ml)**

Sample preparation: Dissolve the protein sample into **1X Labeling Buffer**.
(Before labeling, the concentration of protein is 2~10 mg/ml) and follow the instruction below.

**Concentrations lower than 2 mg/ml will greatly decrease the efficiency of the reaction.

Protein Labeling：

Add the reagents as following into the new tubes. (200 µl tube)

Reagents	Volume (µl)
Protein Sample (2~10 mg/ml)	18
Dye Working Solution	2
Total Volume	**20**

Protein Labeling steps:

1. Add 18 µl **Protein sample** into new 200 µl tube and gently mixing
2. Add 2 µl **Dye Working Solution** and gently mixing
3. Incubate the reaction for **1 hour at room temperature** *(cover by the aluminum foil to avoid the light)*
4. Store protein sample at -20℃**(Avoid the light)**

**** The recommendation molar ratio between Dye and Sample is 2:1～10:1**

User Guide of Protein Labeling Kit (Alexa Fluor™ 488) (Cat. No.C104800)

Labeled Protein Treatment：

1. Using **Protein Dilution Buffer (C104505)**to dilute labeled protein sample to proper concentration (Recommend: Dilute protein sample 200 ~ 500 times)
   *Protein Dilution Buffer (C104505) is included in the Protein Cartridge Kit
2. **Before analyzing**, the protein sample needs to heat **at 100℃ for 5 minutes**. After sample cooling down to room temperature, follow the instruction to analyze the protein samples.

Protein Standard Treatment:

1. Thaw the BenchMark™ Fluorescent Protein Standard at room temperature
2. Use 1:9(v/v) ratio to dilute the Protein Standard by **Protein Dilution Buffer (C104505)**
   *Protein Dilution Buffer (C104505) is included in the Protein Cartridge Kit
3. **Before analyzing**, the protein standard needs to heat **at 100℃ for 5 minutes**. After sample cooling down to room temperature, follow the instruction to analyze the protein samples.

Preparation of Protein Standard mixed with lower marker：

*Use Alexa Fluor™ 488 dye as lower marker to do alignment with labeled protein.

1. Use 1:199(v/v) ratio to dilute the **Dye Stock Solution (10 mg/ml)** by DMSO.
2. Use 1:99(v/v) ratio to dilute the **Dye Solution From Step 1** by 1X Labeling Buffer.
3. Add the reagents as following into the new tubes. (200 µl tube)

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

4. **Before analyzing**, the protein standard needs to heat **at 100℃ for 5 minutes**. After sample cooling down to room temperature, follow the instruction to analyze the protein samples.

Separation Buffer Preparation:

Separation buffer (1X): **5X Protein Separation buffer (C104501-5X)**, d2H_2O as diluent.

Add 1X Separation Buffer into 4 wells of Buffer Tray.

*Buffer height should be equal to the groove of wells.

*5X Protein Separation buffer (C104501-5X) is included in the Protein Cartridge Kit

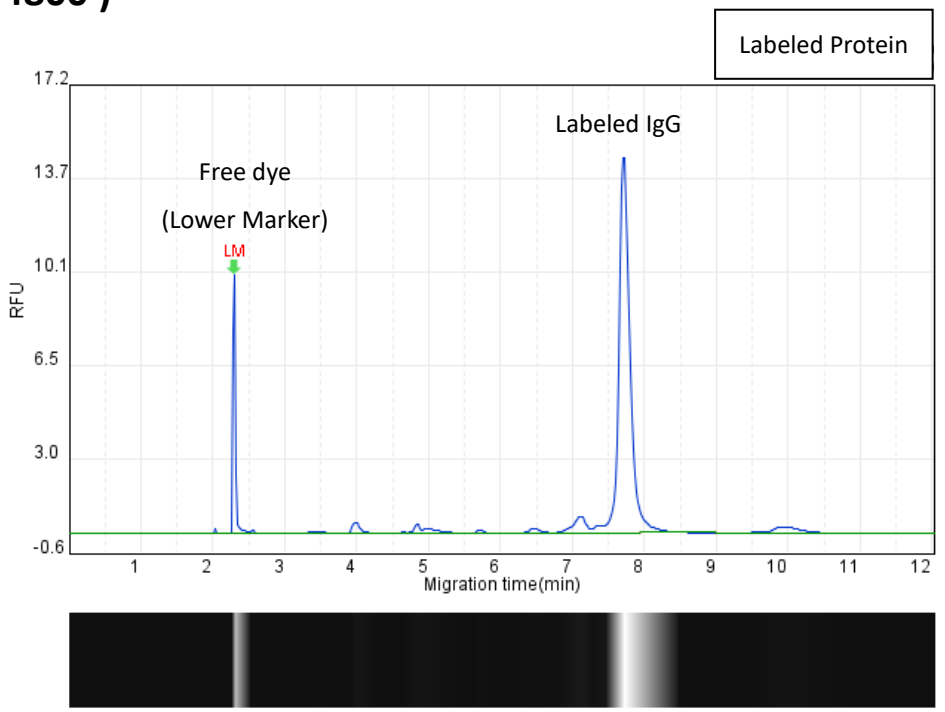
Sample Analysis:

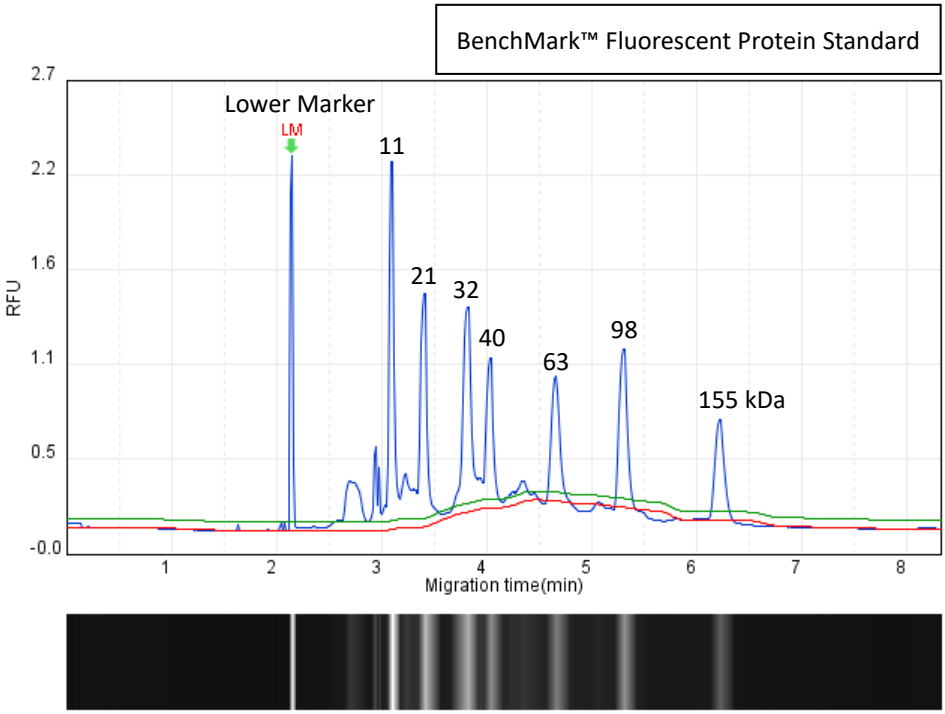
Use the following method to do analysis.

Method	Description
P-4-10-04-1200	Sample Injection 4kv 10s Separation 4kv 1200s

Recommended sample injection duration:
For labeled protein: 1~5s
For protein standard: 10s

User Guide of Protein Labeling Kit (Alexa Fluor™ 488) (Cat. No.C104800)





- **Labeled Protein and Protein Standard should be analyzed by the Protein Cartridge kits(C105121/C105221/C105821) (100 Runs/Cartridge)**