CellMosaic™

CellMosaic, Inc.
10A Roessler Road
Woburn, MA 01801
USA

Phone: 781-463-0002
Fax: 781-998-4694
Email: info@cellmosaic.com
Website: www.cellmosaic.com

AqT® Fluor 750 Protein Labeling Kit (Surface Amines) (CM86256x1 and CM86256x3) User Reference Guide

Contents

CellMosaic, Inc.
10A Roessler Road
Woburn, MA 01801
USA

Phone: 781-463-0002
Fax: 781-998-4694
Email: info@cellmosaic.com
Website: www.cellmosaic.com

Important Notes & Contact Information

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Director of Licensing
c/o CellMosaic, Inc.
10-A Roessler Road, Woburn, MA 01801.

Phone： 781-463-0002
E-mail: info@cellmosaic.com

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Kit Components

Commercial fluorescent dyes are often highly hydrophobic, so dye-labeled proteins can aggregate and exhibit fluorescence quenching at high dye loading due to fluorophore stacking, reducing signal. The AqT® Fluor 750 protein Labeling Kit (Surface Amines) uses CellMosaic's super-hydrophilic, water-soluble, charge-neutral AqT® linker to improve dye-labeled protein performance by increasing solubility, reducing aggregation and nonspecific binding, and minimizing quenching; it employs a core dye with Ex/Em 750/780 nm, and the AqT® spacer helps maintain protein function and produce brighter, more stable conjugates with improved signal intensity.

This kit provides materials to label 6.67 to 20 nano-mole (nmol) of one (CM86256x1) or three (CM86256x3) protein samples (≥20 KDa) with AqT® Fluor 750 acid. The following Table lists Components and storage temperatures for CM86256 Kits; Buffers and tubes are supplied sterile.

⚠ Upon receipt, please remove **Box 1** and store it in a freezer at or below -20°C.
Store **Box 2** (Part number: CM89000) in a refrigerator at 2-8°C.

	Name	Part #	Quantity (CM86256x1)	Quantity (CM86256x3)	Storage condition
Box 1	AqT® Fluor 750 Acid 20 nmol (**red label**)	CM86000	1 unit	3 units	-20°C
Box 2 Part #: CM89000	Activation Buffer (**blue label**)	CM02089	0.15 mL	0.15 mL	2-8°C
	Dilution Buffer (**Magenta label**)	CM02090	0.4 mL	0.4 mL	
	Labeling Buffer (**orange label**)	CM02001	4 mL	12 mL	
	Storage Buffer (1 x PBS buffer with stabilizer) (**grey label**)	CM02022.1	20 mL	60 mL	
	Vis-NIR Measurement Buffer (**black label**)	CM02091	1 mL	1 mL	
	Centrifugal Filter Devices	CM03CD010A	1	3	
	Collection Tubes for Filter	CM03CT0	2	6	
	Desalting Column	CM03SG10	1	3	
	1.5 mL Snap Cap Reaction Tube	CM03CT15	1	3	
	2.0 mL Centrifuge Tube with Cap Attached	CM03CT16	1	3	
User Material	Protein (MW: ≥20 KDa)	NOT PROVIDED (User Supplied Material, **6.67–20 nmol protein** needed per reaction)			

Reaction Scale: This protocol is optimized for conjugating 20 nmol of protein per reaction (protein content as measured by UV). If less than 20 nmol of protein are used, refer to the calculations in **Steps A10. C3, D5, and C6** to determine the correct appropriate sample volumes to add at each step.

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Degree of Labeling (DOL) and Adjustment: The target DOL is 3–7 fluorescent dyes per protein. Depending on the molecular weight and properties of your protein, DOL may differ. For lower–molecular-weight proteins or proteins that tend to aggregate, you can reduce the amount of activated AqT® Fluor dye added to the protein in **Step C3**.

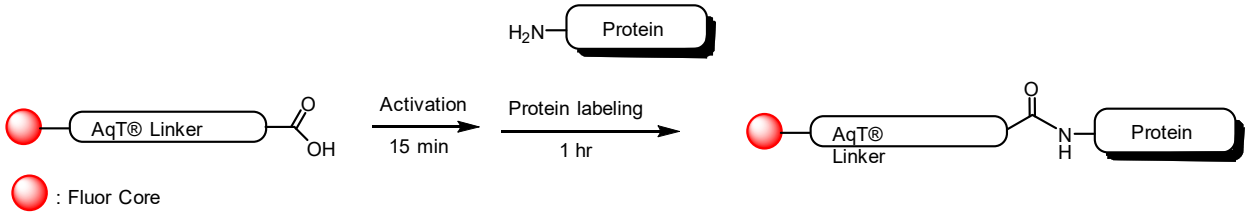
The kit includes a 5-minute UV-Vis-NIR assay buffer and a protocol to estimate the fluorescent labeling level immediately after labeling.

Safety Information

Warning: Some of the chemicals used may be hazardous. Please read and understand the Safety Data Sheets (SDS), available at CellMosaic.com, before storing, handling, or using any of the materials.

Labeling Chemistry

The kit is designed to label any protein with AqT® Fluor 750 dye (**Scheme 1**) via surface amines on the protein. The user supplies the protein. The kit includes AqT® Fluor 750 acid, which is activated within 15 minutes and then coupled directly to the protein in one step. The labeled product is subsequently purified to remove any unreacted AqT® Fluor 750 acid.



Scheme 1: AqT® fluor protein labeling via amide formation.

Key features of AqT® fluor 750 protein labeling kit (surface amines):

- **Optimized for near-infrared imaging:** Features a Fluor 750 core dye (Ex 750 nm / Em 800 nm), ideal for IR-range applications.
- **Advanced AqT® linker technology:** Utilizes CellMosaic's proprietary, super-hydrophilic, water-soluble, charge-neutral AqT® linker to minimize fluorophore stacking and protein aggregation.
- **High dye loading with retained protein properties:** Typical degree of labeling (DOL): 3–5 dyes per protein. No increase in apparent MW (hydrodynamic volume) or aggregation.
- **All-inclusive kit with validated analytical method:** Provides sterilized buffers and tubes, columns, and filters for fluorescent labeling. Also includes all buffers and methods needed for concentration measurement and loading determination.
- **Fast, user-friendly workflow plus optional support:** One-step labeling and purification in ~2 hours (≤1 hour hands-on), with optional post-conjugation services at CellMosaic® for analytical characterization and DOL determination.

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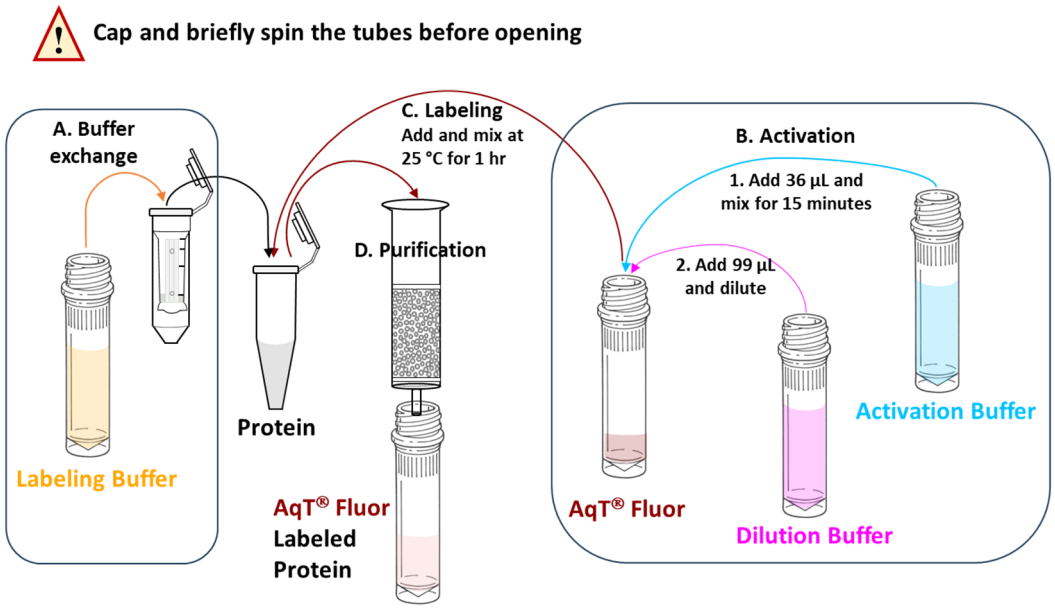
Support

Customer can request a recommendation for the conjugation if the protein has a special feature, a less than 6.67 nmol of protein, or less than 20KDa MW of protein or peptide to be labeled. CellMosaic provides additional accessory tools, such as buffers, standards, and reagents, for protein research. We also offer fee-based support services for customers who require assistance with final conjugate analysis by HPLC and determination of the DOL.

Protocol



Scheme 2. Workflow for preparing AqT® Fluor 750 labeled protein (20 nmol scale reaction).

Read before you start: AqT® Fluor 750–labeled proteins are relatively stable at ambient temperature without aggregation or precipitation; however, the core fluorophore may degrade if the conjugate is stored in solution and repeatedly exposed to air or light. We recommend preparing AqT® Fluor 750–labeled proteins as close to your next experiment as possible to minimize degradation. For long-term storage, store aliquots of the conjugate at **≤ –20°C** for up to a few weeks, or lyophilize to dryness for longer-term storage (months or years). Stability may vary by protein and should be evaluated by **HPLC**.
Requirements for protein: ▪ MW: ≥ 20 KDa ▪ Purity: preferably > 90% pure by gel electrophoresis ▪ Total Amount: 6.67–20 nmol protein content as measured by UV (the accuracy of the protein amount is important factor to obtaining an optimized dye labeling level – please refer to the section '**Other Considerations**' in this manual to measure the protein amount)

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1. Lab Instrumentation Needed

- Vortex mixer, centrifuge (preferably refrigerated, 14,000 *g* capable), mini-centrifuge
- Pipettes and tips
- Timer
- Incubator or shaker (e.g., Eppendorf® Thermomixer 5350)
- Personal protection equipment (lab coat, safety glasses, and chemical-resistant nitrile gloves)

2. Preparation of Protein Samples for Labeling

Items needed: Centrifugal Filter Devices (CM03CD010A), Collection Tubes, Labeling Buffer (CM02001, Orange label), 1.5 mL Snap Cap Reaction Tube (CM03CT15), and clean centrifuge tubes (not provided in the kit).

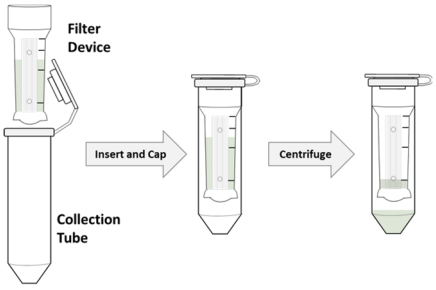
Total amount of protein used for the conjugation is 20 nmol per reaction (protein content measured by UV).

Calculation: Amount of protein (mg) = Molecular Weight (MW) of protein x 0.00002

> **Reaction Scale:** If less than 20 nmol of protein is used, refer to the calculations in **Steps A10. C3, D5, and C6** to determine the correct appropriate sample volumes to add at each step.

A1. Insert the Filter Device into one of the provided collection tubes (see image). Prepare the protein according to its starting condition:

- ✓ **Lyophilized protein**: Dissolve the protein in 500 µL of deionized water and transfer the entire contents to the Filter Device. Cap the device.
- ✓ **protein in < 500 µL buffer**: Transfer the protein directly to the Filter Device, then add Labeling Buffer to bring the total volume to 500 µL. Cap the device.
- ✓ **protein in >500 µL solution**: Transfer up to 500 µL of the protein solution into the Filter Device and cap. Repeat Steps **A2–A4** until the entire sample has been processes. For the final refill (Step A5), add Labeling Buffer to bring the total volume to 500 µL.



A2. Place the capped Filter Device into the centrifuge rotor with the cap strap facing the center. Counterbalance with a similar device.

A3. Centrifuge at 14,000 x g for 10 minutes (preferably at 4°C) to concentrate the sample to **< 100 µL.** *Note: Spin time may vary; A 500 µL sample typically concentrates to ~40 µL after 10–20 minutes (e.g. ~ 8 minutes in an Eppendorf 5417R).*

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A4. Remove the Filter Device and separate it from the collection tube. Transfer the filtrate to a clean centrifuge tube (not provided). **Save the filtrate until all experiments are complete.**

A5. Reinsert the Filter Device into the collection tube. Add 400–450 µL of Labeling Buffer to bring the total volume to 500 µL. Cap and centrifuge at 14,000 x g to concentrate the sample to **< 100 µL**. Transfer and save the filtrate as in Step A4.

A6. Repeat **Step A5** two additional times (total of three buffer exchanges).

A7. Take the **1.5 mL Snap Cap Reaction Tube** from the kit and label the tube. Place it on a balance and tare to zero. Transfer the concentrated protein from the Filter Device to the tube.

A8. Add 200 µL of Labeling Buffer to the Filter Device to rinse. Gently mix with a pipet tip and transfer the rinse to the tube from **Step A7.**

A9. Repeat **Step A8** once.

A10. Place the tube back on the balance and record the total volume by weight (1 µL ≈ 1 mg)**.** Add Labeling Buffer to bring the final sample volume to **615 µL.** Cap the tube.

Calculation 1 for Less Protein Amounts:

$$Final\ volume\ in\ Step\ \boldsymbol{A10}\ (\mu L) = Protein\ (nmol)\ \times 30.75$$

A11. Vortex the protein sample for 30 seconds, then briefly spin it down.

3. Activation of AqT® Fluor 750 for Labeling

Items needed: AqT® Fluor 750 Acid 20nmol (CM86000, red label), Activation Buffer (CM02089, blue label)

B1. Briefly centrifuge the tubes containing **AqT® Fluor 750 Acid 20 nmol** (red label) and **Activation Buffer** (blue label) before opening.

B2. Transfer **36 µL** of **Activation Buffer** into the **AqT® Fluor 750 Acid** tube. Vortex for 30 seconds until the solid is fully dissolved and then spin down.

B3. Incubate at 25 °C in the dark (wrap the tube with alumina foil if necessary) for 15 minutes.

Start Time: _____ End Time:_____

Once Activated, AqT Fluor 750 acid is not stable and cannot be stored; it must be used within **1 hour**.

Tip for mixing or incubating: You may use a nutator, shaker, vortex, or incubator shaker for mixing. If using end-to-end nutating, ensure the centrifuge tube is properly capped. If none of this equipment is available, place the centrifuge tube on the bench and mix manually by pipetting every 20 minutes.

4. Protein Labeling

Items needed: Activated AqT® Fluor from **Step B3**, protein solution from **Step A11,** and Dilution Buffer (CM02090, magenta label).

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C1. Briefly spin the tube containing activated AqT® Fluor solution and **Dilution Buffer** (magenta label) before opening.

C2. Transfer **99 µL** of **Dilution Buffer** into the **activated AqT® Fluor** tube. Vortex for 30 seconds to mix and then spin down

C3. Transfer the entire **activated AqT® Fluor** solution into the tube containing the protein from **Step A11**. When adding the AqT® Fluor solution, insert the pipette tip into the protein solution and slowly dispense the AqT® Fluor solution while gently swirling to ensure uniform mixing.

Calculation 2 for Less Protein Amount:

$$Volume\ of\ AqT®Fluor\ Solution\ to\ add\ (\mu L) = Protein\ (nmol)\ \times 6.75$$

Dispose of any unused activated AqT® solution as a liquid chemical waste.

How to Adjust the DOL: The target DOL is 3–7 fluorescent dyes per protein. For BSA tested to date, this protocol usually yields an average DOL of 5.5. For proteins with a molecular weight <50 kDa or proteins that tend to aggregate, we recommend reducing the amount of activated AqT® Fluor dye added to the protein to 70% of the calculated amount for your initial experiment. Once you have the initial DOL, you can optimize it by increasing or decreasing the amount of activated AqT® Fluor dye added, based on the amount of your protein.

C4. Cap the tube and incubate at 25°C (room temperature) for 1 hour with gentle mixing. While the reaction proceeds, prepare the desalting column (Steps D1–D3).

Start Time: _____ End Time:_____

5. Purification of Fluorescent Labeled Protein

Items needed: Desalting Column (CM03SG10), Storage Buffer (CM02022.1, 1x PBS with stabilizer), 2.0 mL Centrifuge Tube with Cap Attached (CM03CT16), Protein Solution from **Step C4**

D1. Securely attach the **Desalting Column** to a support stand, lab frame, or any support rod. Remove the top and bottom caps from the column, allowing the excess liquid to flow through by gravity. Collect the liquid in a flask.

D2. Add 5 mL of **Storage Buffer** to the column and allow it to fully enter the gel bed by gravity flow.

D3. Repeat **Step D2** twice.

D4. Spin the fluorescent-labeled protein solution from **Step C4** before opening the tube. Add the entire protein solution to the column.

D5. Add 250 µL of **Storage Buffer** to the column**,** allowing the liquid to fully enter the gel bed. (***Note:*** This elution buffer does not contain any of your product and can be discarded as waste).

Calculation 5 for Less Protein:

$$Volume\ of\ Storage\ buffer\ in\ Step\ D\mathbf{5}\ (\mu L) = 1000 - Protein\ in\ nmol\ \times 12.5$$

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D6. Place a **2 mL Centrifuge Tube with Cap Attached** under the column. Add 1.25 mL of **Storage Buffer** to the column and collect the eluent by gravity. Allow the buffer to fully enter the gel bed.

Calculation 6 for Less Protein:

$$Volume\ of\ Storage\ buffer\ in\ Step\ D\mathbf{6}\ (\mu L) = 500 + protein\ in\ nmol\ \times 12.5$$

D7. Label the tube as your product and store the conjugate at 4°C. **Ensure all waste is disposed of in accordance with local regulations.**

D8. Determine the protein concentration and DOL using a UV/Vis spectrophotometer (see **Other Considerations**).

C9. If not used immediately, store the AqT® fluorescent protein at or below -20°C for up to few weeks. For long-term storage, aliquot and lyophilize to dryness, and store at or below -20°C.

The Fluorescent Labeled Protein is Ready for Use

Typical result: AqT® Fluor-labeled proteins typically have an average of 3–5 dyes per protein. A typical batch contains ≥80% conjugated products with no detectable unreacted free dye. Typical recovery is ≥80% with no additional aggregation. Results may vary depending on protein properties.

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Other Considerations

1. Concentration Determination for Protein (Unlabeled)

If your protein comes with a buffer that has no UV absorbance at 280 nm, you can measure the UV absorbance prior to starting an experiment.

$$Concentration\ (M)\ of\ protein = \frac{(A280)}{\varepsilon \times L}$$

Where **L** is the UV cell path length (cm) and **ε** is the extinction coefficient of your protein ($cm^{-1}M^{-1}$)

If your protein comes with a buffer that does absorb at 280 nm, determine the concentration in **Step A6** after exchanging into Labeling Buffer, assuming **95%** recovery after buffer exchange.

2. Concentration and DOL Determination for AqT® Fluor Labeled Protein

This kit targets an average of 3–5 dyes per protein. For BSA tested to date, the average degree of labeling (DOL) is typically close to 4. The analytical method below is designed to estimate experimental DOL and conjugate concentration using a simple UV measurement.

Please note that fluorescent dyes can exhibit complex UV absorbance behavior, and results may vary with pipetting technique. If you require an experimental DOL value, you may submit your sample to CellMosaic for DOL estimation by HPLC. For the most accurate results, submit the sample to CellMosaic or a third-party service for MS analysis.

Protocol

Sample Preparation (for UV-Vis-NIR reading)
Prepare **100 µL** of each solution in a clean centrifuge tube according to the table below.

Solution	AqT® Fluor Labeled Protein (from Step D7)	Storage Buffer CM02022.1 grey label	Vis-NIR Meas. Buffer CM02091 black label	Measurement Wavelength
S1 (20x dilution)	5 µL	95 µL	—	280 nm
UV Buffer Control	—	100 µL	—	280 nm
S2 (20x dilution)	5 µL	—	95 µL	750 nm
NIR Buffer Control	—	5 µL	95 µL	750 nm

UV-Vis-NIR Measurements (Standard 1 cm path length UV quartz cuvette; 100 µL sample volume)

- Measure the UV absorbance of **S1** at 280 nm (**A280**) using **UV Buffer Control** as the blank.
- Measure the NIR absorbance of **S2** at 750 nm (**A750**) using **NIR buffer Control** as the blank.

Calculations

Absorbance ratio
Calculate the UV absorbance ratio (R) using the following formula.

$$R = {}^{(A750)}\Big/_{(A280)}$$

DOL estimate (reference only)
Estimate the average DOL (n) using the following formula:

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$$DAR = \frac{(\text{Ɛ} \times R)}{(200000 - 5800 \times R)}$$

Concentration (reference only)

Calculate the concentration of the diluted sample:

$$Concentration\ (\mu M) = \frac{(A280) * 1000000}{L\ (\text{Ɛ}\ + n * 5800)}$$

$$Concentration\ (mg/mL)\ of\ the\ dilute\ sample = \frac{(A280) \times Mw}{L(\text{Ɛ}\ + n * 5800)}$$

Where:

L = UV cell path length (cm).
ε = protein extinction coefficient
Mw = protein molecule weight

Note: The dye contribution is estimated using the extinction coefficient of the free dye; however, this value may differ for the conjugate. Therefore, the concentration calculated here is for reference only. For a more accurate determination, you may send samples to CellMosaic.

3. Recommended Storage Conditions

AqT® Fluor 750 labeled proteins are relatively stable at ambient temperature without aggregation or precipitation; however, the core fluorophore may degrade if the conjugate is stored in solution and repeatedly exposed to air or light. We recommend preparing AqT® Fluor labeled proteins as close to your next experiment as possible to minimize degradation.

For long-term storage, store aliquots of the conjugate at ⩽ −20°C for up to a few weeks, or lyophilize to dryness for longer-term storage (months or years). Stability may vary by protein and should be evaluated by HPLC.

4. Characterization of AqT® Fluor 750 Protein by Reversed Phase HPLC

For biopolymers labeled with very hydrophobic small molecules, such as fluorescent dyes, reversed phase HPLC may be used to assess the extent of the labeling. **Figure 1** shows an example of AqT Fluor 750 labeling of BSA. Using AqT engineering, a high average DOL (5.5) was achieved despite the extreme hydrophobicity of the dye core.

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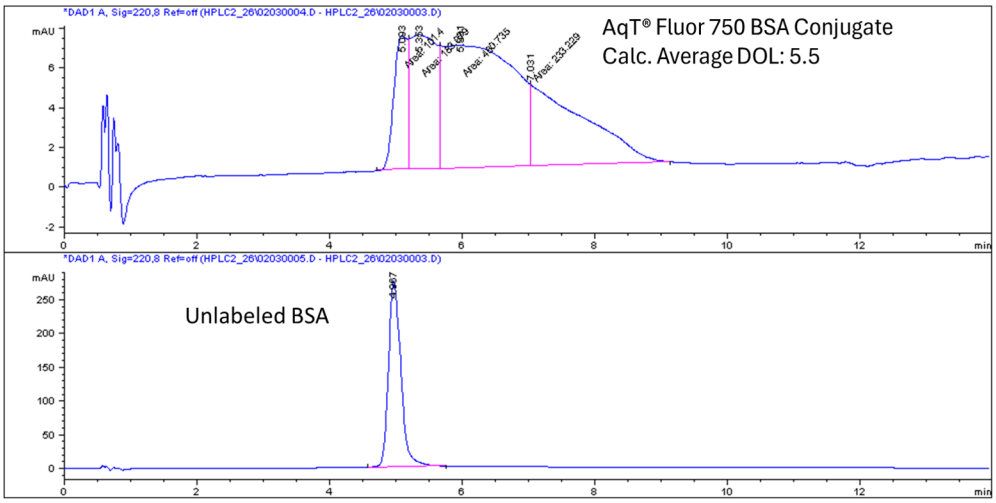
Figure 1: Overlay of reversed phase HPLC profiles of BSA and AqT Fluor 750 Labeled BSA.



5. Characterization of AqT® Fluor Protein by SEC HPLC

Size exclusion chromatography (SEC) separates the conjugates by apparent MW or size in aqueous solution. In general, higher-MW species elute earlier. SEC is also a useful tool for assessing the level of aggregation in a biopolymer. By comparing the SEC profiles of an unlabeled protein and a dye-labeled protein, you can estimate the extent of aggregation in the labeled protein. **Figure 2** shows an example of AqT Fluor 750 of labeling of BSA. AqT Fluor 750 labeled BSA with an average DOL of 5.5 shows a very small shift in apparent MW, and the conjugate has a hydrodynamic volume similar to the native protein. Hardly any additional aggregation is observed after labeling. This confirms that AqT® labeling does not alter protein properties.

Figure 2: Overlay of SEC HPLC analysis of BSA and AqT Fluor 750 labeled BSA (Insert, upper left: UV profiles of individual HPLC peaks).