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 Antibody Labeling Kit (Surface Amines) (CM86248.01x1 and CM86248.01x3, 0.1 mg Scale) User Reference Guide

Contents

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

Important Notes & Contact Information

READ BEFORE USING ANY RESOURCES PROVIDED HEREIN

The information and methods included in this document are provided for information purposes only. CellMosaic provides no warranty regarding performance or suitability for the purpose described. The performance of this kit during labeling may be affected by various factors, including, but not limited to, the purity and complexity of the starting materials, differences in preparation techniques, operator proficiency, and environmental conditions. Sample data if provided, is provided solely for illustrative purposes and as examples of a small dataset used to verify kit performance within the CellMosaic laboratory. Information regarding the chemicals and reagents used in the kit is included where necessary.

For Research Use Only. Not for Use in Diagnostic Procedures.

The information in this document is subject to change without notice. CellMosaic assumes no responsibility for any errors or omissions that may appear in this document. Under no circumstances shall CellMosaic be liable, whether in contract, tort, warranty, or under any statute or on other legal theory, for special, incidental, indirect, punitive, multiple, or consequential damages related to or arising from this document, including but not limited to the use of this product thereof.

PATENT and LICENSE RESTRICTIONIS

CellMosaic's products, including but not limited to AqT®, NeIon™, oxLink™ and sxLink™ (collectively, the "Products"), are covered by, or are the subject of, one or more issued patents and pending patent applications, including without limitation: 9,688,663 B2; 8,907,079 B2; 9,511,150 B2; 9,907,854 B2; 9,346,833 B2; 9,518,067 B2; CN104066451B; CN107043339B; CA 2,841,313; EP 2734238 B1; AU2012284055; JP 6240599 B2; and corresponding foreign patents and patent applications.

The purchase of the Product conveys solely a limited, non-exclusive, non-transferable, non-sublicensable license to use the Product **only for the purchaser's internal research and development purposes**. No right or license is granted, either expressly or by implication, estoppel, or otherwise, under any patent, trademark, copyright, or other intellectual property right of CellMosaic, except as expressly stated herein. Any commercial use of the Product is strictly prohibited without a separate written agreement from CellMosaic. Prohibited commercial uses include, without limitation: (a) the sale, lease, licensing, distribution, or other transfer of the Product or any materials derived from or produced using the Product; (b) the sale, lease, licensing, or other grant of rights to use the Product or any materials derived from or produced using the Product; and (c) the use of the Product to perform services for a fee or other consideration for third parties, including without limitation contract research, screening services, or diagnostic or therapeutic applications.

For information regarding commercial licensing or collaboration, please contact:

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

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

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

Kit Configurations and Components

Commercial fluorescent dyes are often highly hydrophobic, so dye-labeled antibodies can aggregate and exhibit fluorescence quenching at high dye loading due to fluorophore stacking, reducing signal. The AqT® Fluor 750 Antibody Labeling Kit (Surface Amines) uses CellMosaic's super-hydrophilic, water-soluble, charge-neutral AqT® linker to improve dye-labeled antibody 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 antibody function and produce brighter, more stable conjugates with improved signal intensity.

The kit is available in multiple configurations for labeling antibodies ranging from 0.1 to 3 mg. Each configuration supports labeling of either one (×1) or three (×3) antibody samples. This user manual provides instructions for labeling 100 µg of antibody from either a single antibody sample (CM86248.01x1) or three antibody samples (CM86248.01x3). For other amounts, please refer to other configurations.

Table 1: Components and storage temperatures for CM86248.01x1 and CM86248.01x3 Kits (for 100 µg of antibody labeling); 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: CM89000.01) in a refrigerator at 2-8°C.

	Name	Part #	Quantity (CM86248.01 x1)	Quantity (CM86248.01 x3)	Storage condition
Box 1	AqT® Fluor 750 Acid 0.667 nmol (**red label**)	CM86000.01	1 unit	3 units	-20°C
Box 2 Part #: CM89000.01	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	
	Centrifugal Filter Devices	CM03CD010A	1	3	
	Collection Tubes for Filter	CM03CT0	2	6	
	Desalting Spin Column	CM03SG50	2	6	
	Collection Tubes for Spin Column	CM03CT9	2	6	
	0.6 mL Snap Cap Reaction Tube	CM03CT14	1	3	
	1.5 mL Centrifuge Tube with Cap Attached	CM03CT13	1	3	
User Material	Antibody (≥100 KDa)	NOT PROVIDED (User Supplied Material, **0.1 mg antibody (0.667 nmol)** needed per reaction)			

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

Degree-of-Labeling (DOL): This protocol is optimized for IgG antibodies with a molecular weight of 150 KDa to achieve an average labeling ratio of 3–5 fluorescent dyes per antibody (20–33.3 nmol dye per mg of antibody). If your antibody has a different MW, the labeling level **per mg** remains the same; however, the molar ratio will change proportionally.

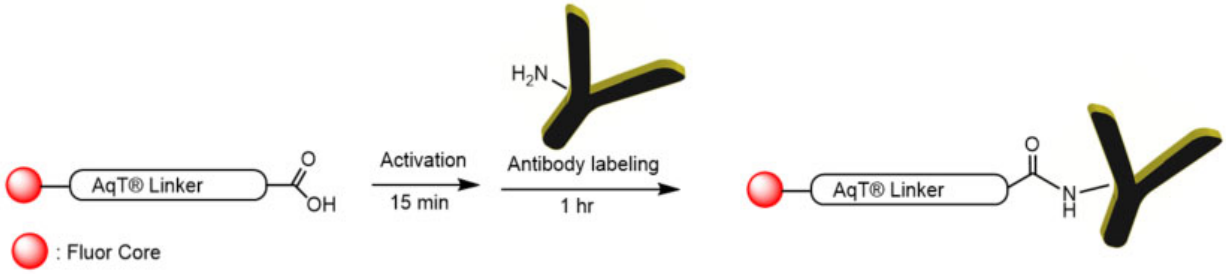
The kit includes a **5-minute UV-Vis-NIR assay buffer** and a protocol to estimate the fluorescent labeling level immediately after labeling.

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



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

Key features of AqT® fluor 750 antibody 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 antibody aggregation.
- **High dye loading with retained antibody properties:** Typical degree of labeling (DOL): 3–5 dyes per antibody. No increase in apparent MW (hydrodynamic volume) or aggregation.
- **Improved stability and lower nonspecific binding:** AqT®-enhanced hydrogen-bonding network improves solubility, helps protect against enzymatic degradation, and minimizes nonspecific interactions with proteins and plastic surfaces.
- **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.

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

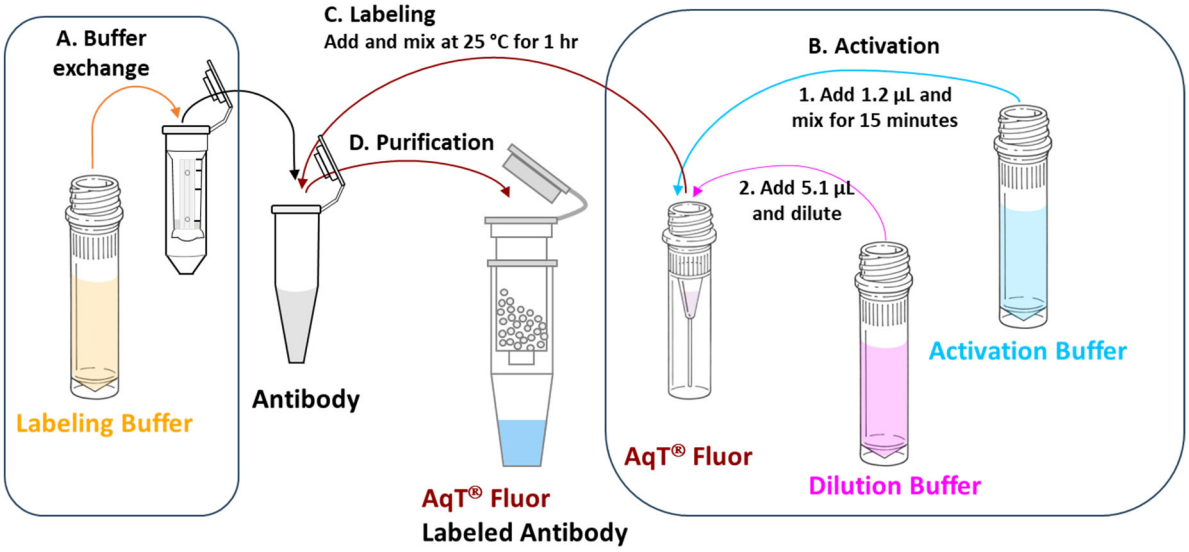
Phone: 781-463-0002
Fax: 781-998-4694
Email: info@cellmosaic.com
Website: www.cellmosaic.com

Support

Customers may request conjugation recommendations if their antibody has unique features. CellMosaic provides additional accessory tools, such as buffers, standards, and reagents, for antibody 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

Cap and briefly spin the tubes before opening



Scheme 2. Workflow for preparing AqT® Fluor 750 labeled antibody (0.1 mg scale reaction).

Read before you start: AqT® Fluor 750–labeled antibodies 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 antibodies 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 antibody and should be evaluated by **HPLC**.

Requirements for antibody:

- MW: ≥ 100 KDa (if MW is lower, please use an alternative kit)
- Purity: preferably > 90% pure by gel electrophoresis
- Total Amount: 100 µg 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)

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

1. Lab Instrumentation Needed

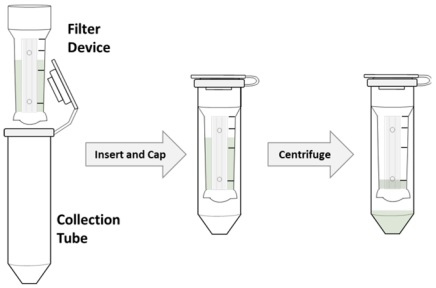
- Vortex mixer, centrifuge (preferably refrigerated, 14,000 *g* capable), mini-centrifuge
- Pipettes and tips
- Timer
- Incubator or shaker (e.g., Eppendorf® Thermomixer 5350)
- Personal protection equipment (lab coat, safety glasses, and chemical-resistant nitrile gloves)

2. Preparation of Antibody Samples for Labeling

Items needed: Centrifugal Filter Devices (CM03CD010A), Collection Tubes, Labeling Buffer (CM02001, Orange label), 0.6 mL Snap Cap Reaction Tube (CM03CT14), and clean centrifuge tubes (not provided in the kit).

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

- ✓ **Lyophilized antibody**: Dissolve the antibody in 500 µL of deionized water and transfer the entire contents to the Filter Device. Cap the device.
- ✓ **Antibody in < 500 µL buffer**: Transfer the antibody directly to the Filter Device, then add Labeling Buffer to bring the total volume to 500 µL. Cap the device.
- ✓ **Antibody in >500 µL solution**: Transfer up to 500 µL of the antibody 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 ~25 µL after 10–20 minutes (e.g. ~ 12 minutes in an Eppendorf 5417R).*

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** one more time (total of two buffer exchanges). Spin at 14,000 x g for 14 minutes to concentrate the solution to **≤30 µL**.

A7. Take the **0.6 mL Snap Cap Reaction Tube** from the kit and label the tube. Place it on a balance and tare to zero. Transfer the concentrated antibody from the Filter Device to the tube, and record the volume by weight (1 µL ≈ 1 mg).

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

A8. Add **5 µL** of Labeling Buffer to the Filter Device to rinse. Transfer the rinse to the tube from **Step A7.** Place the tube back on the balance and record the total volume: **_____ µL.** After combining the concentrated sample (**Step A7)** and the rinse, the total volume should preferably be **30–40 µL**

A9. Vortex the combined antibody sample for 30 seconds, then briefly spin it down.

3. Activation of AqT® Fluor 750 for Labeling

Items needed: AqT® Fluor 750 Acid 0.667 nmol (CM86000.01, red label), Activation Buffer (CM02089, blue label)

B1. Briefly centrifuge the tubes containing **AqT® Fluor 750 Acid 0.667 nmol** (red label) and **Activation Buffer** (blue label) before opening.

B2. Transfer **1.2 µL** of **Activation Buffer** into the **Activation Reagent** 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) without mixing 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**.

4. Antibody Labeling

Items needed: Activated AqT® Fluor from **Step B3**, antibody solution from **Step A9,** and Dilution Buffer (CM02090, magenta label).

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

C2. Transfer **5.1 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 antibody from **Step A9**. When adding the AqT® Fluor solution, insert the pipette tip into the antibody solution and slowly dispense the AqT® Fluor solution while gently swirling to ensure uniform mixing.

C4. Cap the tube and incubate at 25°C (room temperature) for 1 hour with gentle mixing. While the reaction proceeds, prepare the spin column (Steps D1–D2).

Start Time: _____ End Time:_____

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.

5. Purification of Fluorescent Labeled Antibody

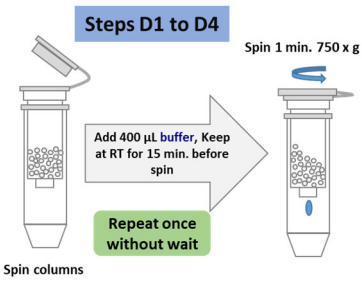
Items needed: Desalting Spin Column (CM03SG50), Storage Buffer (CM02022.1, 1x PBS with stabilizer), 1.5 mL Centrifuge Tube with Cap Attached (CM03CT13), Antibody Solution from **Step C4**.

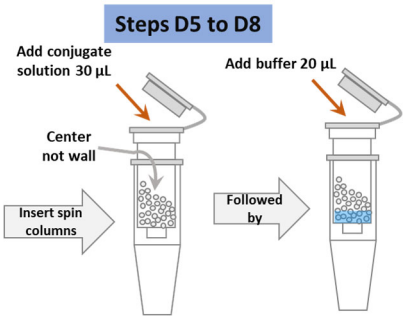
D1. Take out two **Desalting Spin Columns** and remove the bottom red cap. Centrifuge the columns for 1 min at 750 x *g* before opening the top cap.

D2. Apply 400 µL of Storage Buffer (gray label) to the top-center of the resin in each column. Allow the resin to swell at room temperature for 15 min.

D3. Centrifuge for 1 min at 750 x *g* and discard the flow-through.

D4. Repeat **Steps D2–D3** once. After adding Storage Buffer, centrifuge immediately (do not wait) and discard the flow-through.





D5. Place each spin column into a clean 1.5 mL collection tubes.

D6. Spin the fluorescent-labeled antibody from **Step C4** to ensure no liquid remains in the cap before opening. Add Storage Buffer to bring the total volume to **60 µL**.

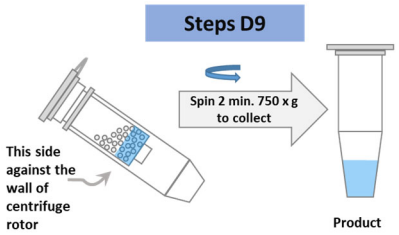
D7. Slowly apply **30 µL** of the fluorescent-labeled antibody solution (from **Step C4**) to the top-center of the resin bed in each column (**2 x 30 µL** total), taking care not to disturb the resin bed.

D8. Rinse the sample tube with **40 µL** of Storage Buffer, then apply **20 µL** of Storage Buffer to the top-center of the resin in each spin column, bringing the total volume in each column to **50 µL**.

The resin bed may partially detach and shift during centrifugation, resulting in an uneven bed or a "pillared" appearance. To prevent issues, apply both the sample and the subsequent PBS buffer **slowly to the center** of the resin bed, avoiding runoff down the sides. Allow the conjugate solution to fully enter the resin before adding the PBS buffer. Do not touch the resin bed with the pipette tip.

D9. Orient each spin column so that the **higher side** of the resin bed faces the **outer wall** of the centrifuge rotor and the **lower side** faces the **center**. Spin for **2 min** at 750 x *g* to collect the eluate.

D10. Transfer and combine the eluates from the two collection tubes into the provided **1.5 mL Centrifuge Tube with Cap Attached**. Dispose of waste follow local regulations.



D11. Determine the antibody concentration using a UV/Vis spectrophotometer (see **Other Considerations**).

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

D12. If not used immediately, store the AqT® fluorescent antibody 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 Antibody is Ready for Use

Typical result: AqT® Fluor-labeled antibodies typically have an average 20–33.3 nmol dye per mg of antibody, which corresponds to an average of 3–5 dyes per IgG antibody (MW 150KDa). A typical batch contains ≥80% conjugated products with no detectable unreacted free dye. Typical recovery is ≥65% with no additional aggregation. Results may vary depending on antibody properties.

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

Other Considerations

1. Concentration Determination for Antibody (Unlabeled)

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

$$Concentration\ (mg/mL)\ of\ Ab = \frac{(A280)}{L \times 1.4}$$

Where **L** is the UV cell path length (cm).

If you know the extinction coefficient (EC) and accurate molecular weight (MW) of your antibody, replace 1.4 with the appropriate conversion factor (EC/MW) based on your antibody's EC and MW.

If your antibody 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 Antibody

This kit targets an average 20–33.3 nmol dye per mg of antibody, which corresponds to an average of 3–5 dyes per IgG antibody (MW 150KDa). For standard human IgG (e.g., a trastuzumab biosimilar) 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 Ab (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

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

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

$$R = {(A750)}/{(A280)}$$

DOL estimate (reference only)

Estimate the average DOL (n) using the following formula:

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

Concentration (reference only)

Calculate the concentration of the diluted sample:

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

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

Where:

L = UV cell path length (cm).
ε = antibody extinction coefficient (for standard IgG, use 210,000 $M^{-1}cm^{-1}$)
Mw = antibody molecule weight (for standard IgG, use 150,000 Da)

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

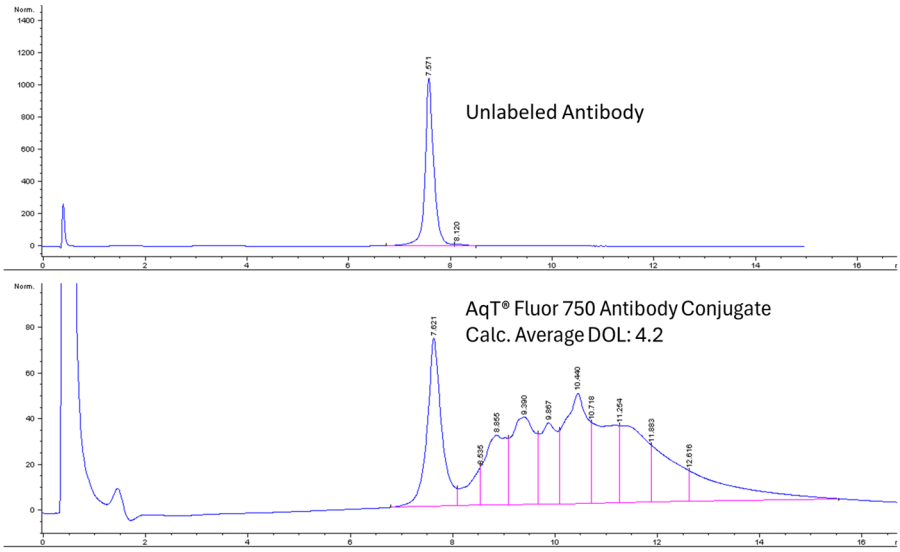
AqT® Fluor 750 labeled antibodies are relatively stable at ambient temperature without aggregation or precipitation; however, the core fluorophore may degrade if the conjugate is stored in solution and repeatedly exposed to air or light. We recommend preparing AqT® Fluor labeled antibodies as close to 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 antibody and should be evaluated by HPLC.

4. Characterization of AqT® Fluor 750 Antibody by HIC HPLC

For biopolymers labeled with very hydrophobic small molecules, such as fluorescent dyes, hydrophobic interaction chromatography (HIC) HPLC may be used to assess the extent of the labeling and the heterogeneity of the labeled molecules. **Figure 1** shows an example of AqT Fluor 750 labeling of a trastuzumab biosimilar (Cat#: CM51000; Human anti-Her2 mAb). Using AqT engineering, a high average DOL (4.2) was achieved despite the extreme hydrophobicity of the dye core.

Figure 1: Overlay of HIC HPLC profiles of the unlabeled antibody and the AqT Fluor 750 antibody conjugate.



5. Characterization of AqT® Fluor Antibody 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 antibody and a dye-labeled antibody, you can estimate the extent of aggregation in the labeled antibody. **Figure 2** shows an example of AqT Fluor 750 labeling of trastuzumab biosimilar (Cat#: CM51000, Human anti-Her2 mAb). AqT Fluor 750 labeling with an average DOL of 4.2 shows no shift in apparent MW, and the conjugate has a hydrodynamic volume similar to, or slightly smaller than, the native antibody. Hardly any additional aggregation is observed after labeling. This confirms that AqT® labeling does not alter antibody properties.

Figure 2: Overlay of SEC HPLC analysis of antibody and AqT Fluor 750 antibody.