Monoclonal Antibodies (Mab's) analysis

Application note Ref : AN 2.001-V2

Antibodies pharmaceuticals are therapeutics that play an important role in controlling a broad range of diseases such as cancer, allergy, inflammation, infectious and autoimmune diseases. Monoclonal antibodies have become a fast growing class of biopharmaceutical products.
To support analytical characterization in process development and quality control of therapeutic antibodies, capillary electrophoresis-sodium dodecyl sulfate (CE-SDS) has been recognized as an important tool in place of SDS-Page because of the ease of use and the ability to automate.
The use of 5-carboxytetramethylrhodamine succinimidyl ester (5-TAMRA.SE) and 3-(2-furoyl)-quinoline-2 carboxaldehyde (FQ) as derivatizing agents improves the sensitivity and the reproducibility of the quantification.

FQ derivatization method

FQ (λmax. Exc.: 480nm; λmax. Emis.: 590nm) is a fluorogenic reagent : it becomes fluorescent only upon reaction with a primary amine.
The method of derivatization is rapid and no purification after labeling is necessary.

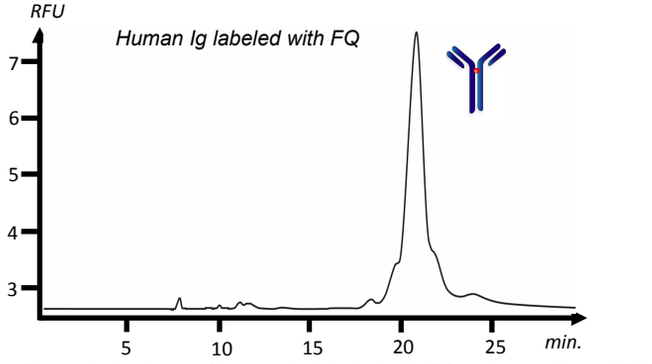

Figure 1: Analysis of human Ig labeled with FQ using a 480nm LED

Instruments: Capillary electrophoresis: Agilent Technologies CE7100
Detector: Picometrics ZETALIF LED with LED 480nm/30nm

Sample: Human Ig labeled with FQ

Labeling: rMAb (2mg/mL) was mixed in 35 µL of 0.1 M citrate-phosphate (pH 6.5), 2% SDS, 10 mM NEM, 1 mM KCN, and 25 nmol FQ; Labeling reactions were incubated for 5 min at 75°C.

Method:
- Capillary: 33cm x 50 µm ID (effective length 19cm)
- Buffer of commercial Beckman SDS kit
- Voltage: -16 kV
- Injection: -10kV, 10s
- Cassette temperature : 40°C

To measure the limit of detection, rMAb sample was spiked with a 28kDa protein size standard at different levels as: 0.2%, 0.5%, 1%, 2%, 5%.

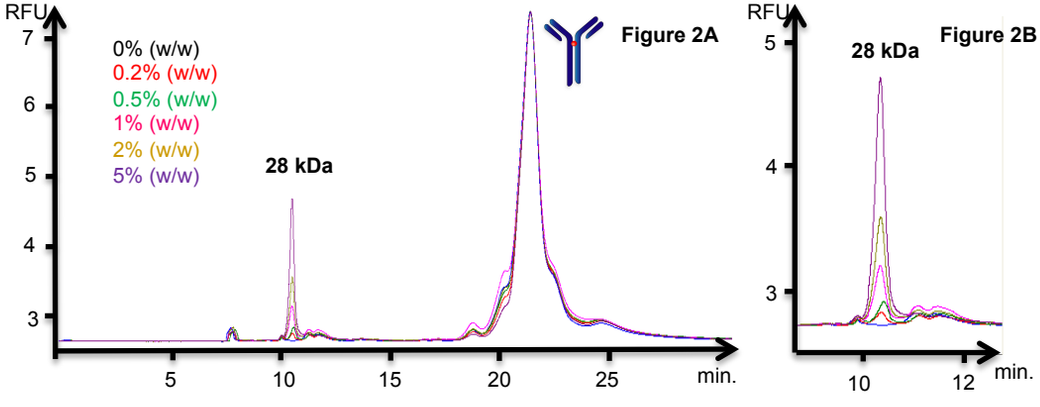

Figure 2 shows that the S/N ratio is 26 at 0.2%.
The extrapolation to a S/N ratio of 3 shows a limit of detection of 0.023% (w/w).

Figure 2A: Separation of human IgG labeled with FQ with five levels of contaminants via CE/L(ed)IF using a 480nm LED
Figure 2B: Extension of the egram of figure 2A between 9 and 12 minutes.

5-TAMRA.SE derivatization method

5-TAMRA.SE (λmax. Exc.: 550nm; λmax. Emis.: 590nm) is commonly used as a labeling reagent of MAb's for fluorescence detection as it increases the sensitivity and maintains the profile of the analyte species.
Figure 3 shows the analysis of human Ig using two LEDs : 480nm and 530nm. Optimization of the excitation wavelength (530nm instead of 480nm) magnifies the S/N ratio by a factor 18.

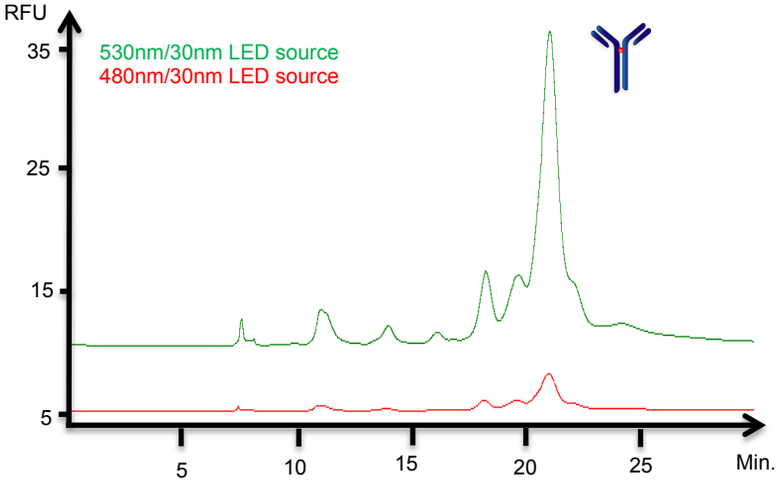

Figure 3: Analysis of human Ig labeled with 5-TAMRA.SE using a 480nm and 530nm LEDs

Instruments: Capillary electrophoresis: Agilent Technologies CE7100
Detector: Picometrics ZETALIF LED with LED 480nm/30nm and 530nm/30nm

Sample: Human Ig labeled with 5-TAMRA.SE

Labeling: rMAb samples (2mg/mL) were buffer exchanged into 0.1 M sodium bicarbonate, pH 8.3, using a NAP-5 column. 10µL of 5-TAMRA.SE (1.4 mg/mL) dissolved in DMSO was then added to 190 µL of rMAb solution and the resultant mixture incubated for 2 h at 30°C. After incubation, 190 µL of the antibody-dye conjugate was loaded onto a second NAP-5 column and collected in 700 µL of 0.1 M sodium bicarbonate, pH 8.3. Nonreduced SDS-rMAb conjugates were prepared by mixing 100 µL of the rMAb-dye conjugate and 100µL of the CE-SDS sample buffer.

Method: same as FQ's method

	FQ derivatization	5-TAMRA.SE derivatization	
Wavelength (nm)	480	480	530
Derivatization method	- Fluorogenic reagent, no purification after labeling; - Rapid method of labeling (5min at 75°C)	- The excess of dye should be removed with NAP-5 column - Long period of time required for labeling and purification (2 hours at 30°C + purification)	
Ratio S/N on the main peak	3 700	1 400	25 800

Conclusion:
This note describes two method of derivatization of IgG with 5-TAMRA.SE and FQ. The CE-L(ed)IF is used to assess the purity and heterogeneity of IgG and its isoforms. The LED presents many advantages : less expensive, less energy consumable, and more stable.

References :
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[iii] Salas-Solano, O.; Tomlinson, B.; Du, S.; Parker, M.; Strahan, A.; Ma, S.
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