Monoclonal Antibodies (mAbs) Analysis

DRUGS

IgG CE-LEDIF
480/530 nm

Application note : AN 2.001

Pharmaceutical Antibodies 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.

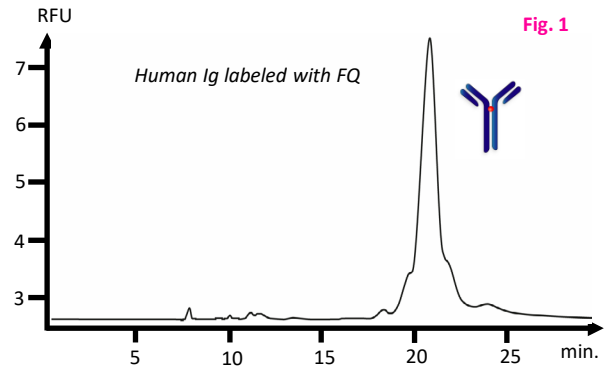

Figure 1 : *Analysis of human Ig labeled with FQ using a 480nm LED*

Instruments	Capillary electrophoresis: Agilent Technologies 7100 CE Detector: Picometrics ZETALIF LED with 480nm/30nm LED
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: 33 cm x 50 µm ID (effective length 19 cm) - Buffer of commercial Beckman SDS kit - Voltage: -16 kV - Injection: -10kV, 10 s - 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%.

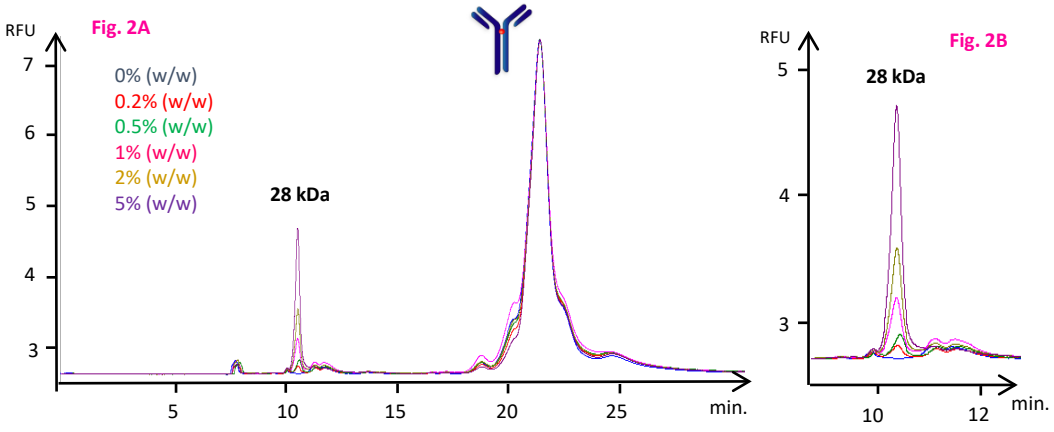

Figure 2 shows that the S/N ratio is 26 at 0.2%.

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/LEDIF 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 mAbs 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.

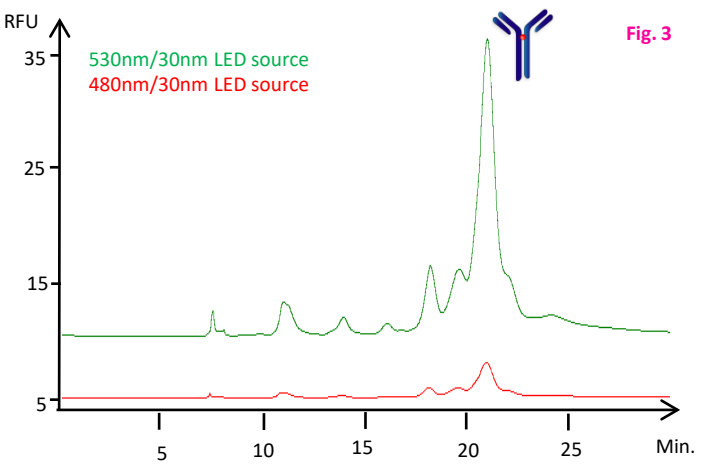

Fig. 3

Instruments	Capillary electrophoresis: Agilent Technologies 7100 CE Detector: Picometrics ZETALIF LED with 480nm/30nm LED and 530nm/30nm LED
Sample	Human Ig labeled with 5-TAMRA.SE
Labeling	rMAb samples (2mg/mL) were 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. Non reduced SDS-rmAb conjugates were prepared by mixing 100 µL of the rmAb-dye conjugate and 100 µL of the CE-SDS sample buffer
Method	Same as FQ method

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

	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	3700	1400	25800

Conclusion:

This note describes two methods of derivatization of IgG with 5-TAMRA.SE and FQ.

The CE-LEDIF system is used to assess the purity and heterogeneity of IgG and its isoforms.

LED light source 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. Anal.Chem. 2006, 78, 6583-6594.