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ATTO-TAG™ CBQCA and ATTO-TAG™ FQ

A-2333 ATTO-TAG™ CBQCA Amine Derivatization Kit
A-6222 ATTO-TAG™ CBQCA derivatization reagent
A-2334 ATTO-TAG™ FQ Amine Derivatization Kit
A-10192 ATTO-TAG™ FQ derivatization reagent

Quick Facts

Storage upon receipt:

- –20°C
- Desiccate
- Protect from light

Introduction

Molecular Probes is pleased to offer the ATTO-TAG™ series of reagents for the ultrasensitive detection of primary amines. Developed by Novotny and collaborators at Indiana University,[1-5] the ATTO-TAG reagents are similar to the important reagents *o*-phthaldialdehyde (OPA), naphthalene-2,3-dicarboxaldehyde (NDA) and anthracene-2,3-dicarboxaldehyde, all of which are also available in high purity form from Molecular Probes.

Principle of ATTO-TAG CBQCA and ATTO-TAG FQ

ATTO-TAG CBQCA (3-(4-carboxybenzoyl)quinoline-2-carboxaldehyde or CBQCA [1]) and ATTO-TAG FQ (3-(2-furoyl) quinoline-2-carboxaldehyde [2]) react specifically with primary amines to form conjugates that can be analyzed by electrophoretic or chromatographic methods. Unlike OPA and NDA, ATTO-TAG CBQCA will also react with hydrophilic peptides and amino sugars. The resulting products of CBQCA — highly fluorescent 7-aza-1-cyano-5,6-benzisoindoles — are maximally excited at 450 nm or by the 442 nm spectral line of the He-Cd laser and have emission maxima at ~550 nm. The products of ATTO-TAG FQ are maximally excited at 480 nm or by the 488 nm line of the argon-ion laser and have emission maxima at ~590 nm. In capillary zone electrophoresis (CZE), the sensitivity of detection of ATTO-TAG CBQCA conjugates should be in the attomole range (10^{-18} moles). The high sensitivity, freedom from background and long-wavelength excitability make these potential reagents for research, diagnostic and forensic applications, including drug analysis (note **A**). The ATTO-TAG reagents can, of course, be used in HPLC and other modes of chromatography with either absorbance or fluorescence detection. The principal limitation to obtaining ultrasensitive detection using chemical derivatization reagents, including the ATTO-TAG reagents, is that relatively high concentrations of the derivatizing reagent are required to obtain adequate kinetics and sufficient modification of the analyte. A recently described technique employs ATTO-TAG FQ for the solid-phase derivatization of dilute solutions (10^{-8} M) of peptides that have been immobilized on Immobilon-CD membranes.[3] This method permits the quantitative derivatization and analysis by capillary electrophoresis of only a few picomoles of the analyte.

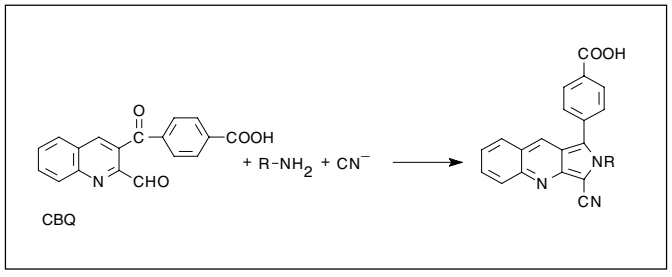

Figure 1. *Reaction of the fluorogenic amine derivatization reagent ATTO-TAG CBQCA.*

Materials

Storage and Handling

Upon receipt, the ATTO-TAG CBQCA and ATTO-TAG FQ Amine Derivatization Kits should be stored frozen at -20°C. The individual CBQCA and FQ derivatization reagents should be stored desiccated at -20°C, protected from light. Stored properly, the ATTO-TAG kits and reagents should be stable for at least six months.

ATTO-TAG CBQCA Amine Derivatization Kit Contents and Solution Preparation (A-2333)

- **ATTO-TAG CBQCA** (MW = 305.3, Component A), 5 mg: A 10 mM solution of CBQCA in dimethylsulfoxide (DMSO) is prepared by dissolving 5 mg CBQCA in 1.64 mL DMSO.
- **Potassium cyanide (KCN)** (MW = 65.1, Component B), >25 mg: Dissolve ~20 mg of KCN in 1.5 mL of deionized water (dH_2O) to give an approximately 0.2 M KCN solution. To obtain a 10 mM working solution for use in *Amino Acids and Peptides* and *Amino Sugars*, dilute the KCN stock

solution 20-fold (5 µL of stock solution + 95 µL of dH_2O). *KCN is hazardous. Handle with extreme care. All solutions should be stored in clearly labeled and tightly sealed containers. Dispose of unused material using methods appropriate for your workplace.*

- **β-cyclodextrin** (Component C), 250 mg: This reagent is sometimes used as an additive in the electrophoresis buffer to enhance the separation of CBQCA–peptide conjugates (see *Peptides*). Weigh out β-cyclodextrin as required for addition to the buffers at a typical concentration of 20 mM (23 mg/mL).

This kit supplies sufficient reagents for 160–320 derivatizations using the conditions described in the protocols. Derivatizations of highly dilute samples ($<10^{-6}$ M) may require a greater excess of reagent to achieve complete reaction.

ATTO-TAG FQ Amine Derivatization Kit Contents and Solution Preparation (A-2334)

- **ATTO-TAG FQ** (MW = 251.2, Component A), 5 mg: A 10 mM solution of FQ is prepared by dissolving the reagent in 2.0 mL methanol.
- **Potassium cyanide (KCN)** (MW = 65.1, Component B), >25 mg: Dissolve ~20 mg of KCN in 1.5 mL of deionized water (dH_2O) to give an approximately 0.2 M KCN solution. To obtain a 10 mM working solution for use in *Amino Acids and Peptides* and *Amino Sugars*, dilute the KCN stock solution 20-fold (5 µL of stock solution + 95 µL of dH_2O). *KCN is hazardous. Handle with extreme care. All solutions should be stored in clearly labeled and tightly sealed containers. Dispose of unused material using methods appropriate for your workplace.*
- **β-cyclodextrin** (Component C), 250 mg: This reagent is sometimes used as an additive in the electrophoresis buffer to enhance the separation of CBQCA–peptide conjugates (see *Peptides*). Weigh out β-cyclodextrin as required for addition to the buffers at a typical concentration of 20 mM (23 mg/mL).

This kit supplies sufficient reagents for 200–400 derivatizations using the conditions described in the protocols. Derivatizations of highly dilute samples ($<10^{-6}$ M) may require a greater excess of reagent to achieve complete reaction.

ATTO-TAG CBQCA Derivatization Reagent (A-6222)

The individual ATTO-TAG CBQCA reagent (MW = 305.3) is supplied in a unit size of 10 mg. A 10 mM solution of CBQCA in dimethylsulfoxide (DMSO) is prepared by dissolving the 10 mg of CBQCA reagent in 3.28 mL DMSO.

ATTO-TAG FQ Derivatization Reagent (A-10192)

The individual ATTO-TAG FQ reagent (MW = 251.2) is supplied in a unit size of 10 mg. A 10 mM solution of ATTO-TAG FQ can be prepared by dissolving the 10 mg of FQ reagent in 4.0 mL methanol.

Storage of Stock Solutions

The stock solutions of ATTO-TAG CBQCA or ATTO-TAG FQ should be stored at -20°C in a capped vial. The stock solution must be thoroughly thawed and mixed before use. A working supply of solution may be kept at room temperature and replaced daily.

Derivatization Procedures

The following protocols are based on the publications of Novotny et al.,[1,2,4-7] and constitute general guidelines for the use of the reagent. Most of the protocols have been developed using CBQCA. Protocols that use ATTO-TAG FQ should be similar.

Amino Acids and Peptides

For the rapid and complete derivatization of amino acids, at least a sixfold molar excess of CBQCA or FQ and a fivefold molar excess of cyanide should be used. For dilute samples, a much larger excess of reagent is required to achieve complete reaction. Typically,[1] a derivatization is carried out by mixing a 2–5 µL aliquot of the amino acid or peptide at a concentration of 10^{-4} to 10^{-6} M with 10–20 µL of 10 mM KCN working solution and 5–10 µL of the 10 mM CBQCA or FQ solution. The mixture is allowed to react for at least one hour at room temperature. A pH of 8.5 to 9.5 has been found to be optimal for reaction of amino acids and smaller peptides (up to four amino acid residues). Larger peptides (nine residues) show no distinct pH optimum.

Amino Sugars

The fluorescence intensity of CBQCA and FQ conjugates with amino sugars is appreciably decreased by high concentrations of CBQCA or cyanide. Optimum results are obtained with a one- to twofold molar excess of CBQCA or FQ and a one- to threefold molar excess of cyanide. The derivatization of amino sugars is performed by mixing an aliquot of the amino sugar with 10–20 µL of the 10 mM KCN working solution and 5–10 µL of the 10 mM CBQCA or FQ solution. The mixture is allowed to react at room temperature for about one hour prior to analysis. A pH of 7 is optimal for reaction of amino sugars with CBQCA and is probably also suitable for FQ derivatization.

Derivatization of Reducing Monosaccharides and Oligosaccharides

Reducing, neutral carbohydrates may be derivatized by CBQCA or FQ following reductive amination with sodium cyanoborohydride in the presence of ammonium ion. Reductive amination is carried out by introducing an excess of 2 M $(NH_4)_2SO_4$ or 4 M NH_4Cl and 0.4 M $NaCNBH_3$ to an aqueous solution of the carbohydrate sample in a screw-cap vial. The tightly sealed vials are shaken to mix well, then placed into a temperature-controlled heating block and kept at 100°C for 100–120 minutes. After completion of the reaction, the solutions are immediately cooled in an ice bath. The mixtures can be used directly for derivatization by CBQCA or FQ as described previously. If desired, the samples may be dried and redissolved (note **B**).

Stability

The cyanoisoindole derivatives of amino acids and peptides are stable in solution for about 24 hours. Those from amino sugars are stable for at least 10 hours. When evaporated to dryness and frozen, the products are stable for at least two weeks.

Separation and Detection

Amino Acids

In simple ionic buffer systems, roughly half of a group of seventeen CBQCA-derivatized amino acids were successfully separated by capillary zone electrophoresis. By adding sodium dodecyl sulfate (SDS) as a micelle-forming agent, most amino acids are adequately resolved. Lysine may present a problem due to double-labeling by CBQCA, leading to fluorescence quenching and spectral changes. Lysine in protein hydrolysates can be analyzed by methylation of the amino side group before hydrolysis. As this also methylates the N-terminus, both methylated and nonmethylated protein samples may be hydrolyzed and derivatized to allow for detection of lysine by comparison.

Detection limits for various CBQCA-derivatized amino acids are found to be in the range of 20–70 attomoles. Glycine is detectable at 1.4 attomoles. The measurements show a linear dynamic range of over three orders of magnitude.

Typical conditions for CZE separation of CBQCA-derivatized amino acids are as follows:

- Capillary: 50 µm i.d. (184 µm o.d.), 104 cm in length (73 cm to detector)
- Mobile phase: 0.05 M TES buffer (pH 7.02), 50 mM SDS, 10 seconds hydrodynamic injection, injection concentration 8.7×10^{-6} M
- Operating Voltage: 25 KV (14 µA)
- Excitation: He-Cd laser, 50 mw at 442 nm
- Emission: 550 nm

These conditions are likely to be different with the uncharged ATTO-TAG FQ.

Peptides

Small peptides (3–4 amino acid residues) and larger ones such as angiotensin derivatives are readily separated as their CBQCA derivatives in a pH 9.5 borate buffer system. Derivatives migrate according to their expected mass-to-charge ratios. Lysine-containing peptides, and proteins in general, may display multiple peaks. The detection limits for a small sampling of model peptides were found to be in the 4.6–13.8 attomole range. The linear dynamic range was at least four orders of magnitude. Significant enhancement of the detection sensitivity and narrower peaks have been obtained for CBQCA–peptide conjugates by incorporating cyclodextrins in the buffer system. The relative fluorescence intensity of larger peptides such as angiotensin derivatives increases nearly 10 times with an increase of β-cyclodextrin concentration from 0–20 mM, while a much smaller increase was observed for a tetrapeptide. Conversely, α-cyclodextrin gives similar fluorescence enhancement for small peptides when present in concentrations of 10–20 mM.

Typical conditions for CZE separation of CBQCA-derivatized peptides are as follows:

- Capillary: 90 cm in length (60 cm to detector), 50 µm i.d.
- Mobile phase: 0.05 borate buffer (pH 9.5) plus 20 mM α- or β-cyclodextrin.
- Operating voltage: 20 KV

These conditions are likely to be different with the uncharged ATTO-TAG FQ.

Amino Sugars

In phosphate buffer, the CBQCA conjugates of D(+)-glucosamine and D(+)-galactosamine show identical electrophoretic mobilities. They are readily separated, however, by addition of borate ion to the buffer. Complete separation has been obtained at borate concentrations of 10 mM.

Dramatic improvements in resolution have been achieved by incorporation of anionic surfactants such as SDS as a buffer additive. The use of SDS concentrations above the critical micelle concentration shifts the separation mode from CZE to micellar electrokinetic chromatography. In this mode, the analyte distribution occurs between the micellar phase and the ionic buffer phase.

Typical conditions for separation of CBQCA-derivatized amino sugars are as follows:

- Capillary: 80 cm in length (50 cm to detector), 50 µm i.d.
- Mobile phase: 20 mM Na_2HPO_4/20 mM borate/50 mM SDS, pH 9.12
- Injections: 10–15 seconds hydrodynamic
- Applied voltage: 16 KV (28 µA)

Notes

[A] Because of their chemical similarity to OPA and NDA, the ATTO-TAG reagents can probably be employed in similar applications.[8] Like NDA,[9] ATTO-TAG CBQCA can potentially be used to determine the amino acid profile of single cells. Moreover, it may be possible to detect amine adducts of ATTO-TAG reagents using electrochemical methods[10] or the chemiluminescent reagent bis-(trichlorophenyl) oxalate (TCPO).[11-13] Other applications include:

- Analysis of amine-containing constituents of human cerebrospinal fluid.[14]
- Detection of enantiomeric separations by capillary electrophoresis.[15]
- Detection of localized chemical modifications on molecular monolayers.[16]

[B] A desalting step may afford a more readily derivatized sample. Following the reductive amination, the sample may be dried and redissolved in methanol, leaving the bulk of the ammonium salts undissolved. Centrifugation should afford a substantially salt-free solution of the amino glycoside.

References

1. Anal Chem 63, 408 (1991); **2.** J Chromatogr 499, 579 (1990); **3.** Electrophoresis 16, 534 (1995); **4.** Anal Chem 63, 413 (1991); **5.** J Chromatogr 519, 189 (1990); **6.** Proc Natl Acad Sci USA 88, 2302 (1991); **7.** J Chromatogr 559, 223 (1991); **8.** J Chromatogr B 659, 85 (1994); **9.** Science 246, 57 (1989); **10.** Anal Chem 61, 432 (1989); **11.** J Chromatogr 511, 155 (1990); **12.** J Chromatogr 464, 343 (1989); **13.** J Pharm Biomed Anal 8, 477 (1990); **14.** Anal Chem 66, 3512 (1994); **15.** Anal Chem 66, 3477 (1994); **16.** Science 268, 272 (1995).

Product List *Current prices may be obtained from our Web site or from our Customer Service Department.*

Cat #	Product Name	Unit Size
A-2333	ATTO-TAG™ CBQCA Amine Derivatization Kit	1 kit
A-6222	ATTO-TAG™ CBQCA derivatization reagent (CBQCA; 3-(4-carboxybenzoyl)quinoline-2-carboxaldehyde)	10 mg
A-2334	ATTO-TAG™ FQ Amine Derivatization Kit	1 kit
A-10192	ATTO-TAG™ FQ derivatization reagent (FQ; 3-(2-furoyl)quinoline-2-carboxaldehyde	10 mg

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