Methyl 10-(2,5-Dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-9-methoxy-3-oxo-3*H*-benzo[*f*]chromene-2-carboxylate (MMBC)

Product No. HC 9080

$$\begin{array}{c|c} & & & & \\ & & & \\ & & & \\ & & & \\ H_3CO & & & \\ \end{array}$$

 $C_{20}H_{13}NO_7$ Mol. Wt.: 379.32 **25 mg = 65.9** µmol

Useful in the detection and determination of thiol-containing proteins, enzymes, and peptides. MMBC, which is essentially nonfluorescent, reacts rapidly with thiols at neutral pH to afford highly fluorescent adducts.

There are several reagents available for the detection and determination of thiols by labeling with a fluorescent reagent. MMBC (Methyl maleimidobenzochromene-carboxylate, also known as Thioglo[®] 1) was developed by Yang and Langmuir for the detection of thiols. The maleimide moiety is susceptible to conjugate addition by thiols, converting MMBC, which is essentially nonfluorescent, into highly fluorescent adducts (Figure 1).

Figure 1. Fluorescent adduct formation from thiols and MMBC.

Advantages of MMBC include:

- MMBC reacts quickly (ca. 1 min) with thiols under neutral conditions (pH 7.0-7.4).
- The fluorescent thiol adducts of MMBC have emission maxima at relatively long wavelengths (513 nm), exhibiting high quantum yields. MMBC itself exhibits very little fluorescence at this wavelength (ca. 40-60 times less fluorescent), thus obviating the separation of unreacted starting material from the mixture.
- MMBC is relatively resistant to hydrolysis at neutral pH. Higher pH's should be avoided (e.g., = 8.0) due to maleimide hydrolysis and nonspecific reactions with amine residues.¹

Useful information: 1,2

- MMBC has a broad absorption band $(\lambda_{max} = 384, \epsilon = 8.86 \text{ x } 10^3)$.
- The thiol adducts of MMBC also show an absorption band at $\lambda_{max} = 384$.
- The thiol adducts of MMBC have an emission maximum at 513 nm.
- MMBC is soluble in DMSO, DMF, and acetonitrile. Dilute the stock solution with neutral buffer before use.
- For information on the use of this reagent and an evaluation of its utility vs. other thiol-detection reagents, see Tyagarajan and coworkers.¹
- Additional information regarding uses of MMBC is available in the literature.⁴

Literature

- 1. Review: Tyagarajan, K.; Pretzer, E.; Wiktorowicz, J. E. *Electrophoresis*, **2003**, *24*, 2348-2358. (http://utmbcare.com/brf/PDF/Electrophoresis2003.pdf)
- 2. Yang, J.-R.; Langmuir, M. E., J. Heterocyclic Chem. 1991, 28, 1177-1180.
- 3. ThioGlo is a registered trademark of Covalent Associates, Inc.

4. (a) Trievel, R; Collazo-Santiago, E.; Couture, J.-F. U.S. Pat. Appl. Publ. (2007), 2007224655 A1. (b) Mi, Z.; Hong, B.; Mirnics, Z. K.; Tyurina, Y.Y.; Kagan, V.E..; Liang, Y.; Schor, N.F. Cancer Chemotherapy and Pharmacology 2006, 57(3), 357-367. (c) Sharov, V. S.; Dremina, E. S.; Galeva, N. A.; Williams, T. D.; Schoeneich, C. Biochemical Journal 2006, 394(3), 605-615. (d) Collazo, E.; Couture, J.-F.; Bulfer, S.; Trievel, R. C. Analytical Biochemistry 2005, 342(1), 86-92. (e) Yakovlev, A. A.; Gulyaeva, N. V. Biomeditsinskaya Khimiya 2004, 50(4), 390-397. (f) Fabisiak, J. P.; Sedlov, A.; Kagan, V. E. Antioxidants & Redox Signaling 2002, 4(5), 855-865. (g) Shaw, C. A.; Pasqualotto, B. A.; Curry, K.; Kim, S. U.; LeCompte, K. A.; Langmuir, M. E. Journal of Neuroscience Methods 1999, 93(1), 21-26. (h) Hua, S.; Fabris, D.; Inesi, G. Biophysical Journal 1999, 77(4), 2217-2225. (i) Yang, J.R.; Langmuir, M. E. Journal of Heterocyclic Chemistry 1991, 28(5), 1177-80.