DABCYL Plus™, an Excellent Substitute for DABCYL and DABSYL in FRET-Based Biological Applications

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Quick facts about DABCYL PlusTM acid and its derivatives

Molecular Weight: 377.42 (Acid, Cat# 81803); 474.49 (SE, Cat# 81804); 499.54 (C2 Maleimide, Cat# 81805); 419.51 (C2 Amine, Cat# 81823)
Absorption Maximum: 437 nm in MeOH; 485 nm in H2O
Emission Maximum: none
Storage Conditions: desiccated, refrigerated and protected from light

INTRODUCTION

In recent years, fluorescence resonance energy transfer (FRET) has been widely used in various biological applications. FRET is the transfer of the excited state energy from the initially excited donor (D) to an acceptor (A). The donor molecules typically emit at shorter wavelengths that overlap with the absorption of the acceptor. The process is a distance-dependent interaction between the electronic excited states of two molecules *without emission of a photon*.[1-5]

In a FRET system, the donor and acceptor molecules can be the same or different. In most applications, they are different dyes. FRET can be detected either by the appearance of sensitized fluorescence of the acceptor or by the intensity ratio change of donor/acceptor (if the acceptor is fluorescent), or the fluorescence decrease of the donor. Probes incorporating fluorescent donor/non-fluorescent acceptor (*e.g.* DABCYL) combinations have been developed primarily for detection of proteolysis, nucleic acid hybridization[7-9], receptor/ligand interactions[10], distribution and transport of lipids[11,12], membrane potential sensing[13] and cyclic AMP detection[14].

PHYSICAL AND CHEMICAL PROPERTIES OF DABCYL PLUS™ QUENCHERS

DABCYL is the abbreviation of 4-(**d**imethyla**m**inoazo)**b**enzene-4-**c**arboxy**l**ic acid. In some literature, DABSYL [4-(**d**imethylamino-**a**zo)**b**enzene-4'-**s**ulfony**l** chloride] is misused as 'DABCYL'. DABCYL has been the most popular acceptor for developing FRET-based nucleic acid probes and protease substrates. However, its extremely high hydrophobicity and resultant poor water solubility have limited its use in the development of sensitive fluorogenic FRET probes. We have often noted that its high hydrophobicity tends to reduce enzyme affinity for DABCYL containing FRET substrates. DABCYL Plus™ has been developed to address this limitation. As shown in the following figures, DABCYL Plus™ retains spectral properties similar to those of DABCYL. This feature enables researchers to keep all assay settings similar to DABCYL's probes to which they are accustomed. In addition, DABCYL Plus™ has 80 times greater water solubility than DABCYL. We have used DABCYL Plus™ to develop various protease substrates. In some cases, it has demonstrated greatly improved enzyme performance. This may be partly due to the slightly red-shifted absorption spectra that overlap better with the emission spectrum of EDANS, the popular donor paired with DABCYL in FRET applications. Additionally, the absorption spectrum of DABCYL Plus™ is environment-sensitive as in the case of DABCYL dyes. For example, in water, the spectrum of DABCYL Plus™ is red-shifted ca. 40 nm compared to that in methanol.

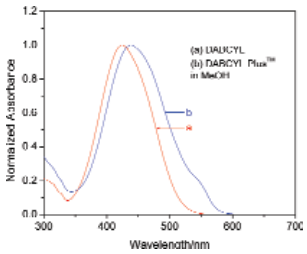


Figure 1. Absorption spectra of DABCYL and DABCYL PLUS™ in MeOH.

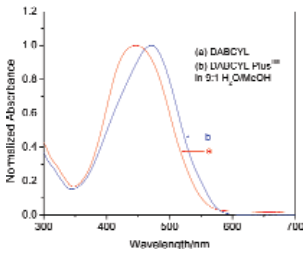
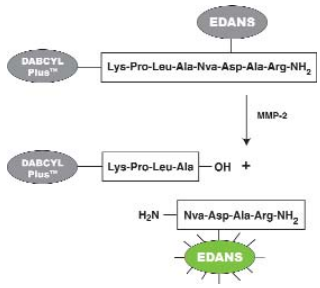


Figure 2. Absorption spectra of DABCYL and DABCYL PLUS™ in 9:1 H_2O/MeOH.

APPLICATIONS OF DABCYL PLUS™ IN DEVELOPMENT OF FLUOROGENIC MMP SUBSTRATES

The matrix metalloproteinases (MMPs) constitute a family of zinc-dependent endopeptidases that function within the extracellular matrix. These enzymes are responsible for the breakdown of connective tissues and are important in bone remodeling, the menstrual cycle and repair of tissue damage. While the exact contribution of MMPs to certain pathological processes is difficult to assess, MMPs appear to have a key role in the development of arthritis as well as in the invasion and metastasis of cancer. MMPs tend to have multiple substrates, with most family members having the ability to degrade different types of collagen along with elastin, gelatin and fibronectin. Most MMPs contain three major domains: a regulatory domain (which must be removed before the enzyme can be active), a catalytic domain and a hemopexin domain. The hemopexin domain aids in enzyme binding to certain substrates, although it is not necessary for the catalytic function of the enzyme.



Scheme 1. The MMP-2 cleavage of DabcylPlus-Lys-Pro-Leu-Ala-Nva-Asp(Edans)-Ala-Arg-NH2.

We have used DABCYL Plus™ in the development of a fluorogenic MMP-2 substrate (see Scheme 1). As shown in Scheme 1, the DABCYL Plus™-based FRET peptide is readily cleaved by MMP-2. The MMP-2-induced peptide cleavage generates the fluorescence signal of EDANS that is proportional to MMP-2 activity and reaction time.

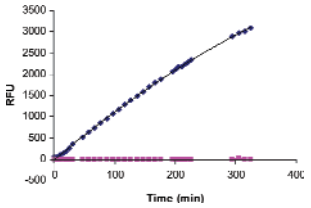


Figure 3. The proteolytic cleavage of FRET substrate by MMP-2.

50 µM FRET MMP-2 substrate (DABCYL Plus™-Lys-Pro-Leu-Ala-Nva-Asp(Edans)-Ala-Arg-NH2) is incubated with 4 nM MMP-2 (blue square) or without MMP-2 (pink square) at room temperature (background). The fluorescence signal is recorded on a fluorescence microplate reader at Ex/Em=360±40 nm/460±40 nm. The recording is started as soon as the enzymatic reaction is initiated.

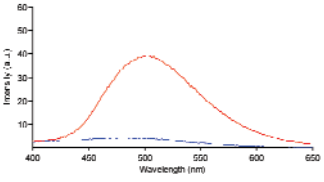


Figure 4. The spectral change during the proteolytic cleavage of FRET substrate by MMP-2.

50 µM FRET MMP-2 substrate (DABCYL Plus™-Lys-Pro-Leu-Ala-Nva-Asp(Edans)-Ala-Arg-NH2) is incubated with 4 nM MMP-2 (red line) or without MMP-2 (blue line) for 24 hours at room temperature. The fluorescence signal is recorded on a fluorescence spectrometer at Ex/Em=340±10 nm/490±5 nm.

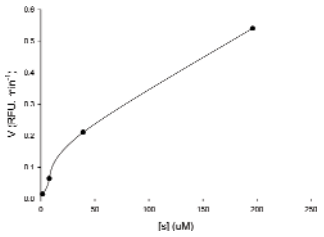


Figure 5. The Michaelis-Menten plots of the enzymatic reaction of MMP-2 with the DABCYL Plus™-derived peptide substrate.

Different concentrations of FRET MMP-2 substrate (DABCYL Plus™-Lys-Pro-Leu-Ala-Nva-Asp(Edans)-Ala-Arg-NH2) are incubated with 4 nM MMP-2 in the buffer containing 0.1 M Tris, pH 7.5, 0.1 M NaCl and 0.05% Brij 35. The initial velocity at each substrate concentration is calculated and plotted in Figure 3.

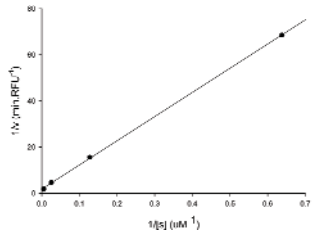


Figure 6. The Lineweaver-Burk double-reciprocal plot of the enzymatic reaction of MMP-2.

The data are converted from Figure 5. The Km is determined to be 56.18 µM.

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