

## Product Information

### 3-Cyano-7-ethoxycoumarin

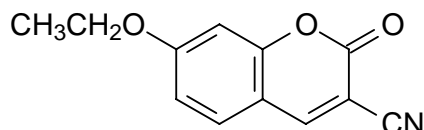
Product Number **C 2612**  
Store at Room Temperature

CAS No. 117620-77-6

Synonym: 7-ethoxy-2-oxo-2H-1-Benzopyran-3-carbonitrile; CEC

#### Product Description

Molecular formula: C<sub>12</sub>H<sub>9</sub>NO<sub>3</sub>  
Formula wt.: 215.2



3-Cyano-7-ethoxycoumarin is fluorogenic substrate suitable for the continuous determination of cytochrome P450 mixed-function monooxygenases. The product of the reaction is the fluorescent compound 3-cyano-7-hydroxycoumarin (Product No. C 2737).

Fluorescence of 3-cyano-7-hydroxycoumarin occurs at neutral pH with excitation and emission at 408 and 450 nm, respectively.<sup>1</sup> Fluorescent reaction product detection is at least 50-fold more sensitive than that of the product of alkyl resorufin oxidation because of greater rate of turnover of CEC.<sup>1,2</sup> The ability to continuously monitor the enzyme reaction at pH 7 is derived from the lower pK<sub>a</sub> of the 3-cyano-7-hydroxycoumarin product compared to that for 7-ethoxycoumarin.<sup>3,4</sup>

CEC is a suitable substrate for three of the five principal cytochrome P450 drug metabolizing enzymes, CYP1A2, CYP2C9, and CYP2C19.<sup>5</sup> It has been used to measure cytochrome P450 mixed-function monooxygenases in rat hepatic microsomal preparations as well as in isolated rat hepatocytes.<sup>1</sup> It was part of a study to examine the expression of CYP2B6 in human liver microsomes, and has been used to characterize Clara and type II cells from rat lung.<sup>6,7</sup>

#### Preparation Instructions

Prepare stock solutions in DMSO. It can also be suspended in phosphate or Tris buffer, pH 7.4-7.5, by sonication.<sup>5</sup>

#### Storage/Stability

Store at room temperature. Protect from light.

#### References

1. White, I. N., A continuous fluorometric assay for cytochrome P-450-dependent mixed function oxidases using 3-cyano-7-ethoxycoumarin. *Anal. Biochem.*, **172**, 304-310 (1988).
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3. Zitting, A., A sensitive liquid chromatographic assay of ethoxycoumarin deethylase with fluorescence detection. *Anal. Biochem.*, **115**, 177-80 (1981).
4. Ullrich, V, and Weber, P., The O-dealkylation of 7-ethoxycoumarin by liver microsomes. A direct fluorometric test. *Hoppe Seylers Z. Physiol. Chem.*, **353**, 1171-1177 (1972).
5. Crespi, C. L., et al., Microtiter plate assays of inhibition of human, drug-metabolizing cytochromes P450. *Anal. Biochem.*, **248**, 188 (1997).
6. Ekins, S., Further Characterization of the Expression in Liver and Catalytic Activity of CYP2B6. *J. Pharmacol. Exp. Ther.*, **286**, 1253-1259 (1998).
7. Martin, J, et al., Characterization of Clara and type II cells isolated from rat lung by fluorescence-activated flow cytometry. *Biochem. J.*, **295**, 73-80, (1993).

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