

IRDye®

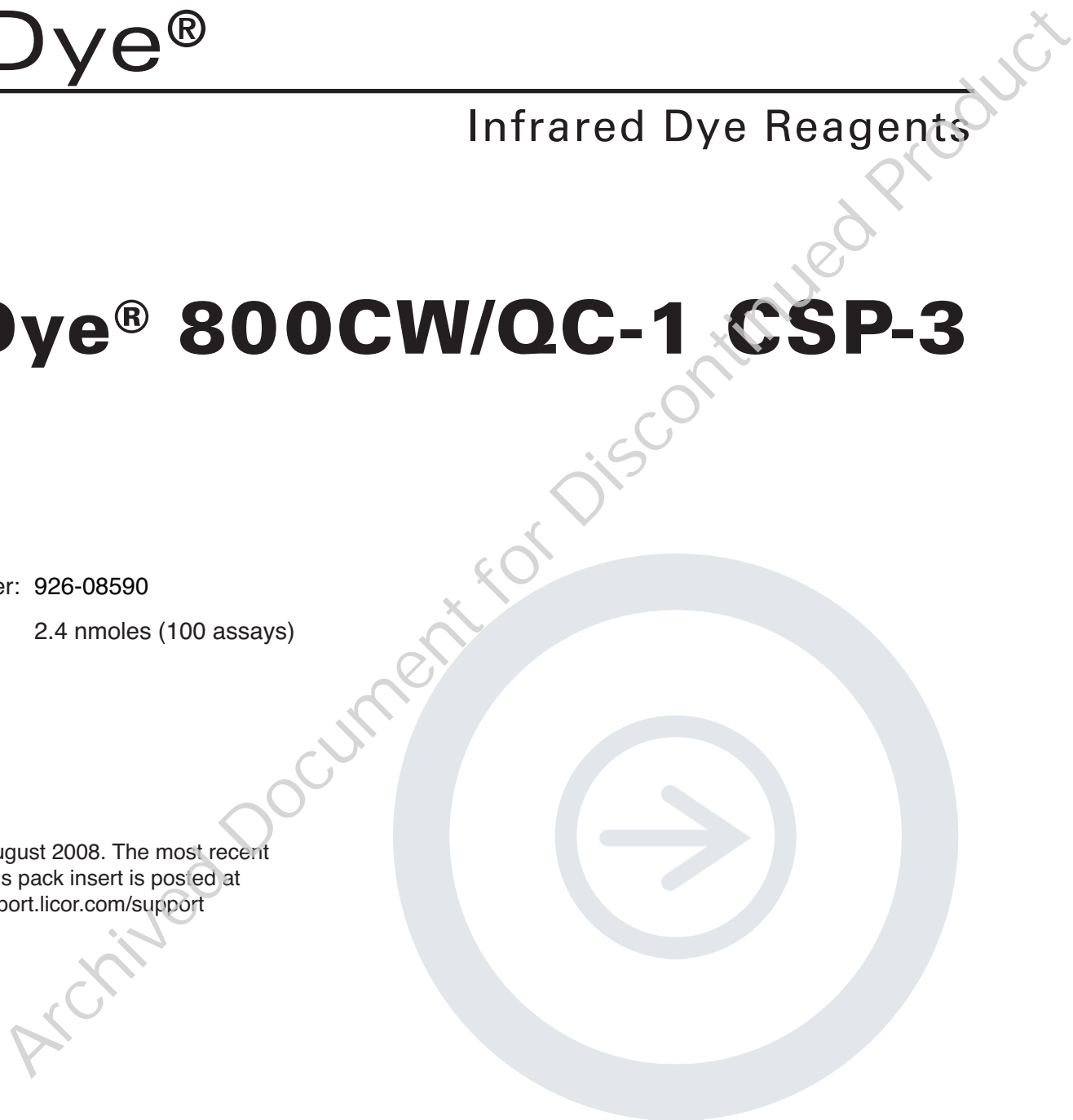
Infrared Dye Reagents

IRDye® 800CW/QC-1 CSP-3

Part Number: 926-08590

Size: 2.4 nmoles (100 assays)

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IRDye® 800CW/QC-1 CSP-3

Sequence: IRDye 800CW-Gly-Asp-Glu-Val-Asn-Gly-Ala-Lys (IRDye QC-1)-COOH

Size: 2.4 nmoles (100 assays for one 96-well plate)

Background

Caspases are a family of intracellular cysteine proteases that are vital in the process of apoptosis (programmed cell death). Caspase-3 functions as the most downstream executioner in the caspase-apoptosis signaling cascade.¹ Activation of Caspase-3 by upstream initiator caspases (Caspases 2, 8, 9) results in the final execution of cell death.² Insufficient or accelerated apoptosis is implicated in a variety of diseases such as cancer, immune system diseases and nervous system disease.³

Description

Caspase-3 fluorogenic peptide substrate is dual labeled with IRDye 800CW and an efficient dark quencher, IRDye QC-1. Cleavage of the fluorogenic peptide substrate between the Asp-Glu amino acids by Caspase-3 restores the fluorescence in a fluorescence resonance energy transfer assay (FRET). The substrate demonstrates a limit of detection as low as 1×10^{-5} Units/mL of Caspase-3 enzyme using human recombinant enzyme (Millipore). The substrate also shows very high sensitivity for measuring endogenous Caspase-3 activity in apoptotic Jurkat cells. The limit of detection in cell-based assays in a 96-well format is 1500 to 3000 cells per well. A broad, linear dynamic range for the relationship between fluorescent intensity and number of apoptotic cells has been demonstrated.*

The substrate is optimized for use with Odyssey® Infrared Imaging System, Aeries™ Automated Infrared Imaging System or other imaging systems with suitable excitation and emission characteristics. Use of near-infrared fluorescence substantially eliminates background fluorescence from assay media.

Excitation max: 774 nm; Emission max: 789 nm.

Purity: >90% by HPLC

Form: Lyophilized solid

Solubility: Sterile water

Use and Storage: Store lyophilized at -20°C for up to 6 months. Reconstitute in 200 µL of sterile water before use. Store reconstituted peptide for up to 3 months at -20°C. Protect from light and moisture. Dilute the reconstituted peptide 1:30 with assay buffer for use with 96-well plate FRET assays.

* For more detailed example of use, see Document #982-09817, "IRDye® 800CW/QC-1 CSP-3 Substrate for Caspase-3 Activity".

References

- 1 Thornberry, N. A.; Lazebnik, Y. "Caspases: enemies within" *Science*, 281, 1312-1316, 1998.
- 2 Slee, E. A.; Adrain, C., Martin, S. J. "Serial killers: ordering caspase activation events in apoptosis" *Cell Death Differ*, 6, 1067-1074, 1999
- 3 Danial, N. N.; Korsmeyer, S. J. "Cell death: critical control points" *Cell*, 116, 205-219, 2004.

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