



Fluo-4 AM Lot 10112a

Method	Specification	Analysis
LCMS	Agilent 1220 Infinity II	
Purity*	≥ 95%	95.0 %
Molecular Ion	<i>Common Peaks</i> 1097.29 ± 0.5 m/z (MH ⁺) 1119.27 ± 0.5 m/z (MNa ⁺) 549.15 ± 0.5 m/z (MH ₂ ²⁺)	<i>Detected Peaks</i> 1097.60 m/z 1119.60 m/z 549.50 m/z
Absorbance Spectrum	Agilent Cary 60 UV-VIS Spectrophotometer	
Longest-Wavelength Absorbance Maximum**	494 ± 3 nm	494 nm
Fluorescence Spectrum	Horiba Jobin Yvon FluoroMax 4 Spectrofluorometer	
Excitation Max.; Emission Max.**	491 ± 3 nm; 516 ± 3 nm	494 nm; 516 nm
¹H NMR Spectrum	Bruker Avance 400	
Peaks and Integrations	Only relevant product peaks — with appropriate chemical shifts and peak integrations — and solvent peaks present	Confirmed
Cell Assay	BioTek Cytation 5 Imaging Reader	
F/F ₀ post-stimulus in relevant biological assay	≥ 3.30	3.34

*Column: Agilent Infinity Lab Poroshell 120 ECC18, 3.0 x 50 mm, 2.7 μm C₁₈, UV-Vis Diode Array Detector: 254 nm, Single Quad MS Detector: ESI Positive; **solvent: High-Calcium Buffer, AM esters hydrolyzed to ion-sensing salt form prior to acquiring spectral data.

Approved by P. Rogelio Escamilla Dec 2019