ION BIOSCIENCES

Certificate of Analysis

Calcein AM

Lot 10622a

Method	Specification	Analysis
LCMS	**Agilent 1220 Infinity II**	
Purity[1]	≥ 95.0%	99.0 %
Molecular Ion[2]	995.3 ± 0.5 m/z $[M+H]^+$ 498.5 ± 0.5 m/z $[M+2H]^{2+}$	995.6 m/z 498.5 m/z
Absorbance Spectrum	**Agilent Cary 60 UV-VIS Spectrophotometer**	
UV-Visible λ_{max}[3]	494 ± 3 nm	493 nm
Fluorescence Spectrum	**Horiba Jobin Yvon FluoroMax Plus Spectrofluorometer**	
Excitation λ_{max} [3] Emission λ_{max}	494 ± 3 nm 514 ± 3 nm	492 nm 513 nm
^{1}H NMR Spectrum	**Bruker Avance 400**	
Peaks and Integrations	Conforms to Structure	Conforms
Cell Assay	**BioTek Cytation 5 Imaging Reader**	
Signal to Background Ratio[4]	≥ 10	99
Imaging Analysis[4]	Observed fluorescence is intracellular, with minimal extracellular fluorescence	Conforms

[1]Column: Phenomenex 00d-4251-E0 Luna C_{18}, 4.6 x 100 mm, 100Å, 3 µm , UV-Vis Diode Array Detector: 254 nm; [2]Single Quad MS Detector: ESI Positive; [3]Solvent: PBS, AM esters hydrolyzed to ion-sensing salt form and then adjusted to and or measured at pH 7.2 prior to acquiring spectral data; [4]Solvent: PBS, λ_{max} Ex/Em: 495/515 nm

David Woody
Quality Manager 4 Feb 2025