



## Fluo-4 K<sup>+</sup> Salt Lot 10212a

| Method  | Specification   | Analysis  |
|---|---|---|
| <b>LCMS</b>   | <b>Agilent 1220 Infinity II</b>   |   |
| Purity*   | ≥ 90%   | 93.7%   |
| Molecular Ion (Acid Form)   | <i>Common Peaks</i><br>737.18 ± 0.5 m/z (MH <sup>+</sup> )<br>759.16 ± 0.5 m/z (MNa <sup>+</sup> )<br>369.10 ± 0.5 m/z (MH <sub>2</sub> <sup>2+</sup> ) | <i>Detected Peaks</i><br>737.50 m/z<br>759.40 m/z<br>369.40 m/z |
| <b>Absorbance Spectrum</b>  | <b>Agilent Cary 60 UV-VIS Spectrophotometer</b>   |   |
| Longest-Wavelength Absorbance Maximum**                                 | 494 ± 3 nm  | 491   |
| <b>Fluorescence Spectrum</b>  | <b>Horiba Jobin Yvon FluoroMax 4 Spectrofluorometer</b>   |   |
| Excitation Max.; Emission Max.***                                       | 494 ± 3 nm; 517 ± 3 nm  | 495 nm; 518 nm  |
| <b><sup>1</sup>H NMR Spectrum</b>                                       | <b>Bruker Avance 400</b>  |   |
| Peaks and Integrations  | Only relevant product peaks — with appropriate chemical shifts and peak integrations — and solvent peaks present  | Confirmed   |
| <b>In Vitro Assay</b>   | <b>BioTek Cytation 5 Imaging Reader</b>   |   |
| F <sub>Stim</sub> / F <sub>Ctrl</sub> in relevant buffer solution assay | ≥ 263.4   | 316.9   |

\*Column: Agilent Infinity Lab Poroshell 120 EC-C18, 3.0 x 150 mm, 2.7 μm, UV-Vis Diode Array Detector: 254 nm, Single Quad MS Detector: ESI Positive; \*\*solvent: MeOH, \*\*\*solvent: High-Calcium Buffer

Approved by P. Rogelio Escamilla Dec 2019

Reanalyzed Apr 2022