ION BIOSCIENCES

# Certificate of Analysis

## Fluo-3 AM

### Lot 10520a

| Method | Specification | Analysis |
|---|---|---|
| | | |
| **LCMS** | **Agilent 1220 Infinity II** | |
| Purity* | ≥ 95% | 97.5% |
| Molecular Ion | *Common Peaks*<br>1129.23 ± 0.5 m/z ($MH^+$)<br>1151.21 ± 0.5 m/z ($MNa^+$)<br>565.12 ± 0.5 m/z ($MH_2^{2+}$) | *Detected Peaks*<br>1129.5 m/z<br>1151.5 m/z<br>566.2 m/z |
| | | |
| **Absorbance Spectrum** | **Agilent Cary 60 UV-VIS Spectrophotometer** | |
| Longest-Wavelength Absorbance Maximum** | 505 ± 3 nm | 507nm |
| | | |
| **Fluorescence Spectrum** | **Horiba Jobin Yvon FluoroMax 4 Spectrofluorometer** | |
| Excitation Max.; Emission Max.** | 506 ± 3 nm; 526 ± 3 nm | 505 nm; 523 nm |
| | | |
| **$^1$H 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 | ≥ 2.79 | 3.43 |

*Column: Agilent Infinity Phenomex 00d-4251-E0 4.6 x 100 mm. 100 angstrom, 3 µm Luna $C_{18}$ (2), 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 A. Vergote Dec 2023