TEF LABS
9415 Capitol View Drive
Austin TX 78747
TEL: 512.280.5223
FAX: 512.280.4997
WEB: www.TefLabs.com
EMAIL: support@teflabs.com

Product Specifications Sheet

Product Name	ASANTE Calcium Red™ sfc (K^+ Salt)
MW	~1000 g/mol
K_d	~300-400 nM
Description	Dark red solid
TLC	C_{18} on 60Å silica, 200 µm thickness
Solvent	7:3 methanol/brine
R_f	0.6
Purity	> 90%
HPLC	Waters™ XTerra® MS-C18, 4.6 x 100 mm
Flow rate	2 mL / min
Solvents	A = 0.01 M triethylammonium phosphate, pH 2.8 B = acetonitrile
Solvent Gradient	35% B for 10 min (isocratic)
Retention Time (minutes)	6.76 minutes
Detector Settings	254 nm 460 nm
Purity	> 90% > 90%
Absorption Spectrum	
Solvent	Methanol
λ_{max}	555 nm
ε	45000 $M^{-1}cm^{-1}$
Excitation Spectra	***(Figure 1)***
Solvent	10mM EGTA, 100mM KCl, 10mM MOPS, pH 7.2
λ_{em}	650 nm
λ_{max}	540 nm
Non-Ratiometric Emission Spectra	***(Figure 2)***
Solvent	10mM EGTA, 100mM KCl, 10mM MOPS, pH 7.2
λ_{ex}	540 nm
λ_{max}	650 nm (emission intensity increases strongly with increasing calcium)
Ratiometric Emission Spectra	***(Figures 3 and 4)***
Solvent	10mM EGTA, 100mM KCl, 10mM MOPS, pH 7.2
λ_{ex}	488 nm
λ_{max}	517 nm (emission intensity decreases slightly with increasing calcium) 650 nm (emission intensity increases strongly with increasing calcium)

TEF LABS
9415 Capitol View Drive
Austin TX 78747
TEL: 512.280.5223
FAX: 512.280.4997
WEB: www.TefLabs.com
EMAIL: support@teflabs.com

Product Specifications Sheet

All titrations were performed with varying proportions of 10 mM K_2EGTA, 100 mM KCl, 10 mM MOPS and 10 mM CaEGTA, 100 mM KCl, 10 mM MOPS at pH 7.2

Figure 1: **Excitation traces of a calcium titration of sfc (K^+ Salt)**

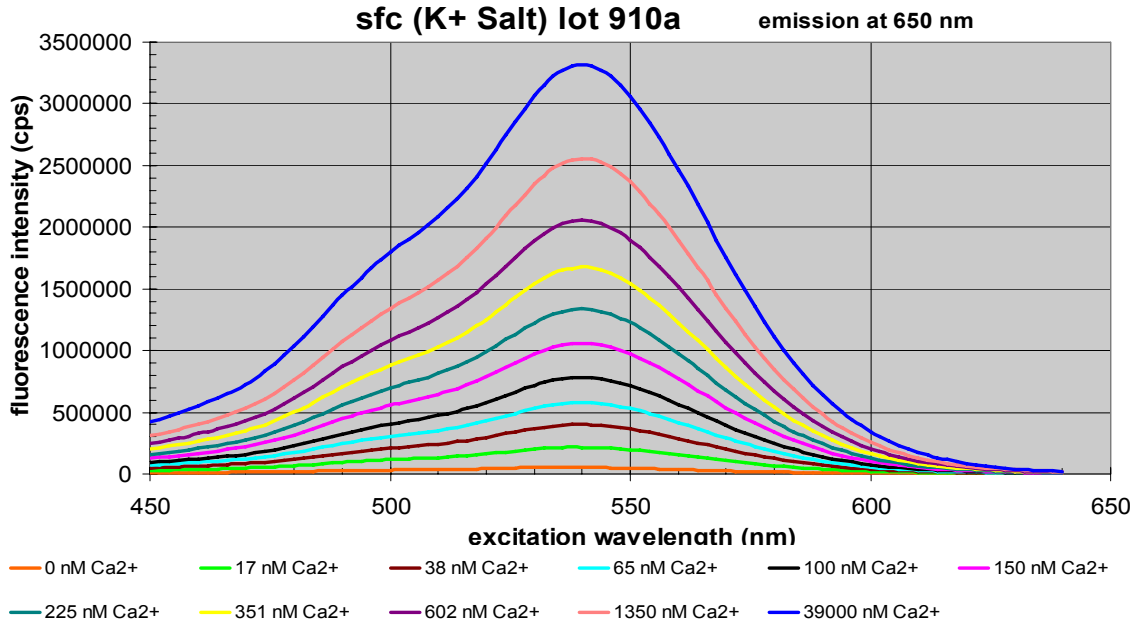
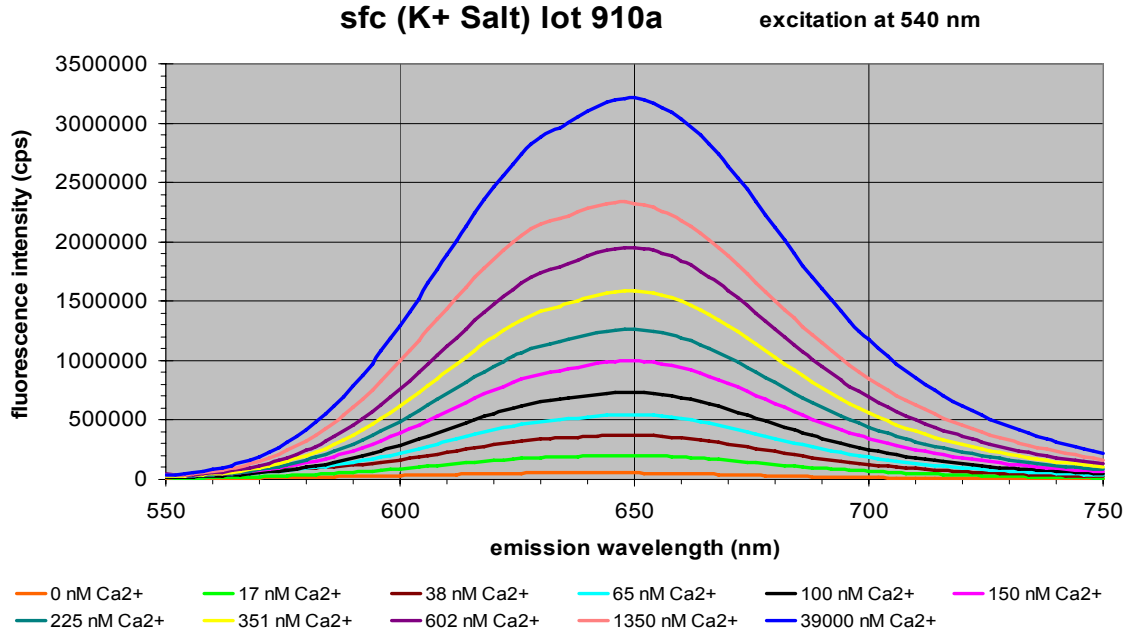


Figure 2: **Non-ratiometric emission traces of a calcium titration of sfc (K^+ Salt)**



TEF LABS
9415 Capitol View Drive
Austin TX 78747
TEL: 512.280.5223
FAX: 512.280.4997
WEB: www.TefLabs.com
EMAIL: support@teflabs.com

Product Specifications Sheet

Figure 3: **Ratiometric emission traces of a calcium titration of sfc (K^+ Salt), lot 910a**

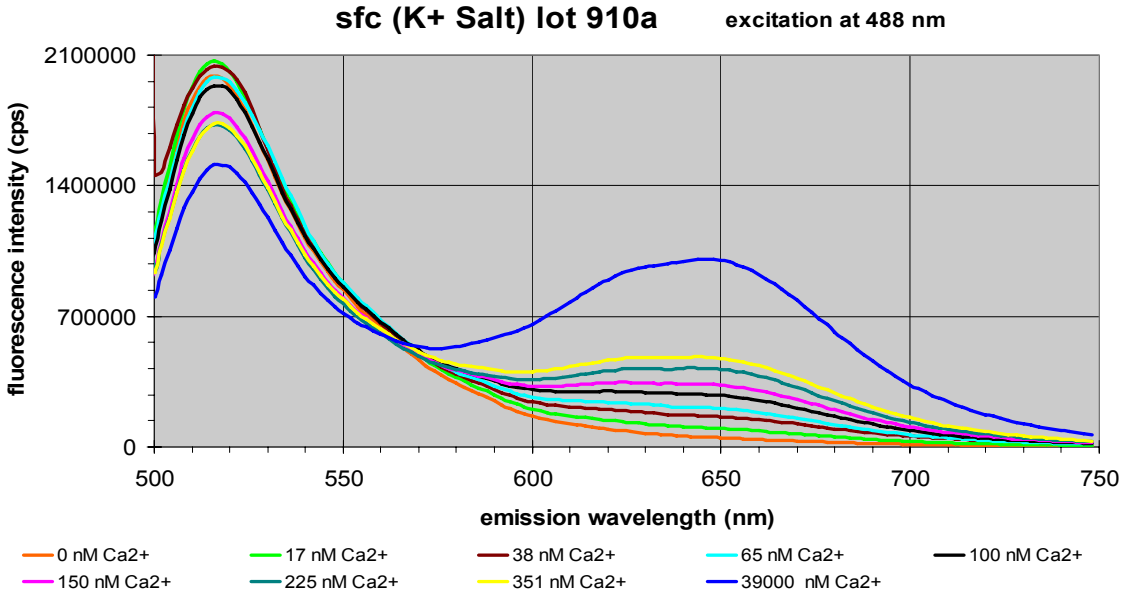
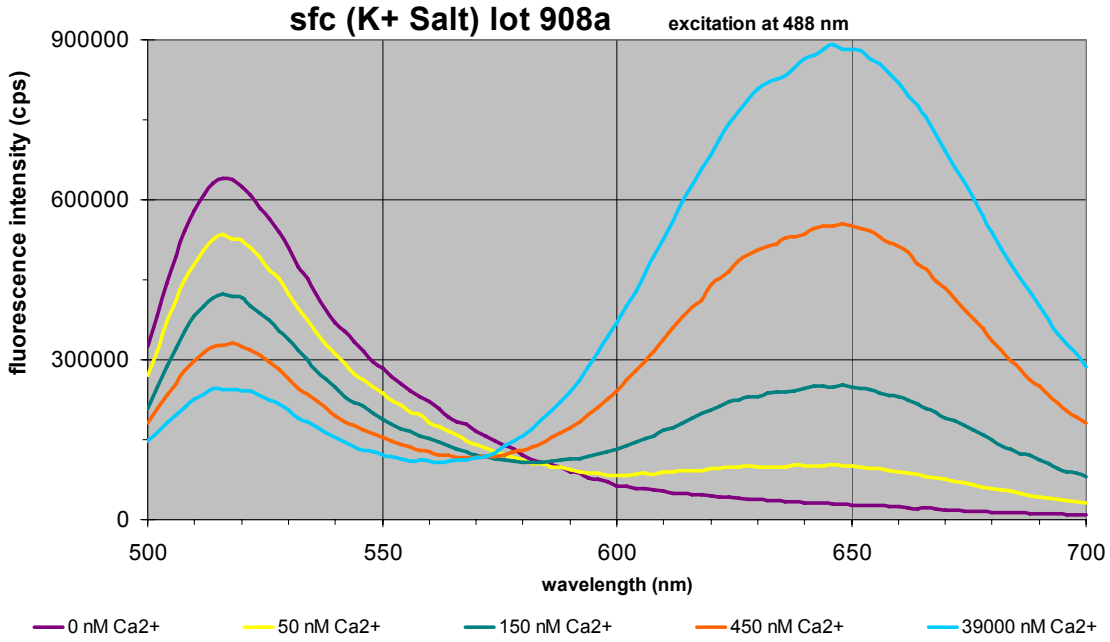


Figure 4: **Ratiometric emission traces of a calcium titration ot sfc (K^+ Salt), lot 908a**



Note: Calibration of sfc (K^+ Salt) in the cell has not been performed yet. Initial results in rat cardiac myocytes and neurons show an attenuated response at ~520 nm, with little to no response to calcium changes. In contrast, calcium changes produce a

TEF LABS
9415 Capitol View Drive
Austin TX 78747
TEL: 512.280.5223
FAX: 512.280.4997
WEB: www.TefLabs.com
EMAIL: support@teflabs.com

strong response at 650 nm. In our hands, as can be noted from the variance in the lots 908a and 910a, we have not yet achieved consistent results for the 517 nm peak, although the 650 nm peak has consistently shown a ~20-fold increase in emission upon calcium saturation.

Note2: Exciting at 488 nm produces only 40% of the emission at 650 nm obtained from 540 nm excitation (*Figure 1*), so that adjustments for the reduced emission (i.e., higher dye concentration or increasing power to the excitation source) may be necessary.