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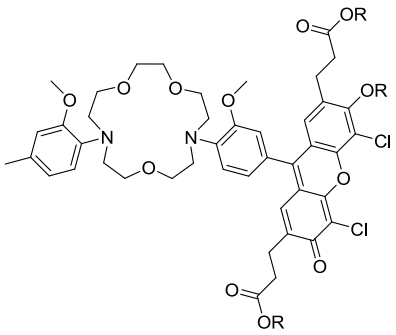
Empowering Research

Asante NaTRIUM Green 2 (**ANG-2**)

ANG-2 is to ***Sodium***

as

Fluo-4 is to ***Calcium***



ANG-2 (TMA+ Salt):
R = NMe_4

ANG-2 (AM):
R = CH_2OCOCH_3

ANG-2 Highlights

- Ex_{max} at 517 nm (532 nm intracellular)[1], two-photon Ex_{max} at 790 nm[1]
- Em_{max} at 540 nm (548 nm intracellular)[1]
- Eosin or JOE filter sets RECOMMENDED
- $F/_{F0}$ of 20 from zero to saturated sodium
- 20 mM/34 mM K_d for sodium (absence/presence of K^+), 39 mM (in astrocytes)[1]
- AM ester loads readily at room temperature
- Little compartmentalization, even distribution throughout the cytosol
- Good retention within cell and resistant to photobleaching at 37 °C
- TMA^+ Salt Product Specifications
- TMA^+ Salt MSDS
- AM ester Product Specifications
- AM ester MSDS

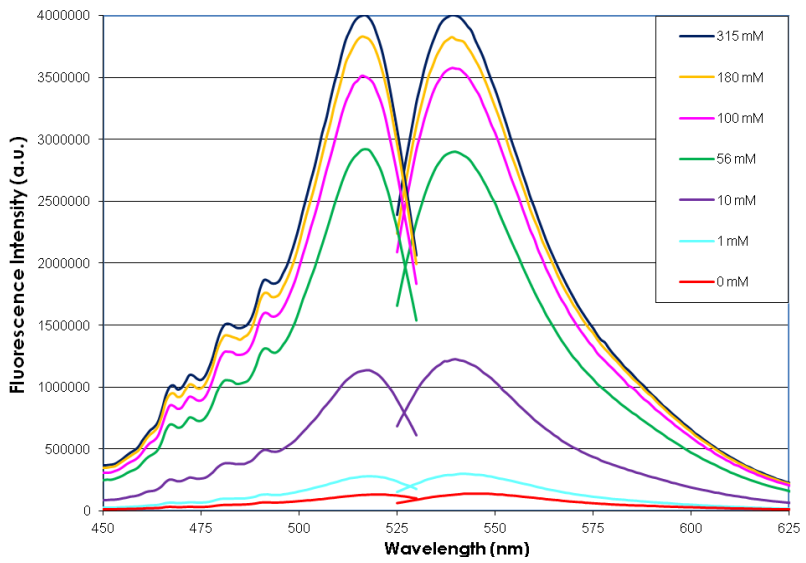
Experimental Results for ANG-2

- [1] Lamy, C. and Chatton, J.Y. **2011** *NeuroImage* **58**(2): 572-8
- [2]O'Donnell, G.T. *et al*. ANG-2 Voltage Gated Sodium Channel Assay Dept. of Exploratory Sciences & Screening, Merck Research Labs, **2011** SLAS Orlando, FL

- Excitation and emission spectra of a titration with sodium chloride
- ANG-2 response to the application of Na^+ ionophore SQI-Pr in REF52 fibroblasts
- Intracellular titration of ANG-2 with NaCl in REF52 fibroblasts
- ANG-2 response to voltage-gated sodium channel stimulation in HEK293 cells

ANG-2 (AM) Loading Protocol

- Acetoxymethyl ester (AM) loading protocol for ANG-2



Excitation and emission spectra for an NaCl titration of ANG-2: To a 3 µM solution of ANG-2 (TMA^+) in 130 mM TMACl, 10 mM MOPS, pH 7.1 were added appropriate volumes of 1M NaCl in 10 mM MOPS, pH 7.1. Excitation was set at 517 nm for the emission scans, and emission was set at 539 nm for the excitation scans. Spectra were corrected for dilution, but not for increasing ionic strength or Cl^- quenching.

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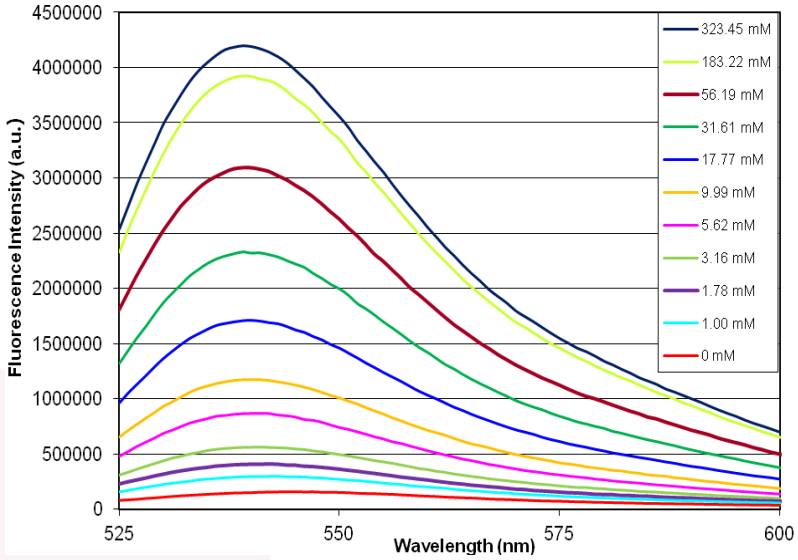

Emission spectra for an NaCl titration of ANG-2: To a 3 µM solution of ANG-2 (TMA^+) in 130 mM TMACl, 10 mM MOPS, pH 7.1 were added appropriate volumes of 1M NaCl in 10 mM MOPS, pH 7.1. Excitation was set at 517 nm for the emission scans. Spectra were corrected for dilution, but not for increasing ionic strength or Cl^- quenching.

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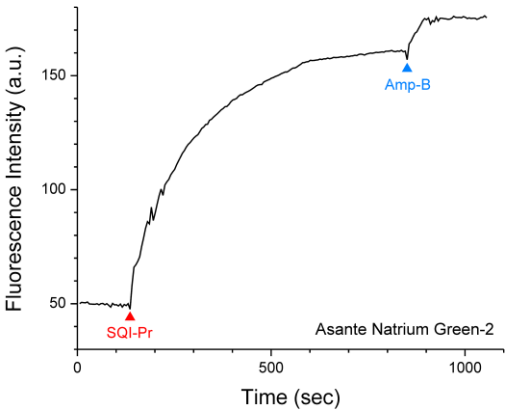
ANG-2 Response to Na^+ Influx Facilitated by SQI-Pr



Plot quantifying the enhanced fluorescence of ANG-2, resulting from increased cytosolic sodium ion concentration provoked by the application of the sodium ionophore SQI-Pr. REF52 fibroblasts loaded with ANG-2 (AM) in HBSS were exposed to 40 µM SQI-Pr, a Na^+ ionophore that promotes Na^+/H^+ exchange across the cell membrane. The fluorescence of intracellular ANG-2 increased steadily, reflecting a rise in intracellular [Na^+]. Further addition of 20 µM amphotericin B, a polyene natural product that increases membrane permeability to all the common monovalent ions, gave a small additional increment of indicator fluorescence, as expected. This experiment demonstrates the utility of ANG-2 as a sodium indicator and the efficacy of SQI-Pr as a sodium ionophore. The response shown is an average from 30 cells.

Experimental results courtesy of Dr. JPY Kao, University of Maryland

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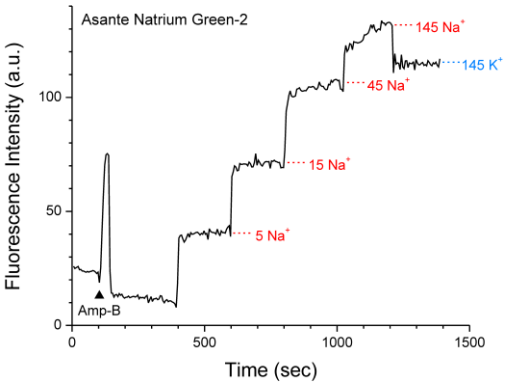
ANG-2 Intracellular Titration with NaCl



Plot quantifying the enhanced fluorescence of ANG-2, resulting from increased cytosolic sodium ion concentration. REF52 fibroblasts loaded with ANG-2 (AM) were maintained in 145 mM N-methyl-D-glucamine (NMG) gluconate. To deplete cells of Na^+ and K^+, the cells were treated with 50 µM amphotericin B, a polyene natural product that increases membrane permeability to all the common monovalent ions. Increments of NaCl were added to raise the sodium concentration in the extracellular medium to various levels (5, 15, 45,and 145 mM). After each increment, as the intracellular and extracellular [Na^+] equilibrated, a corresponding increase in intracellular indicator fluorescence was observed. At the end of the experiment, an aliquot of KCl was added to raise [K^+] to 145 mM. The added K^+ decreased the fluorescence of ANG-2 modestly. The response shown is an average from 25 cells.

Experimental results courtesy of Dr. JPY Kao, University of Maryland

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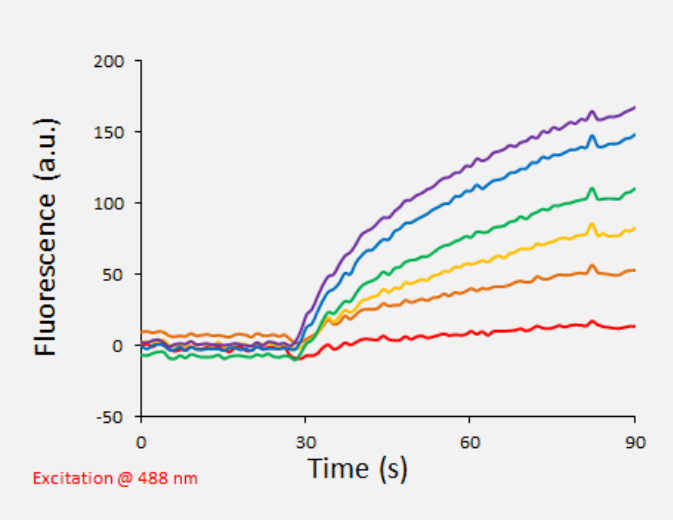
ANG-2 Response to Voltage-Gated Sodium Channel Stimulation



NaV1.3-expressing HEK-293 cells loaded with ANG-2 (AM) were exposed to a concentration series of lidocaine, a voltage-gated sodium channel blocker. The cells were stimulated with 25 mM veratridine in 20 mM HHBS.

Experimental results courtesy of Dr. Dave Weaver, Vanderbilt University

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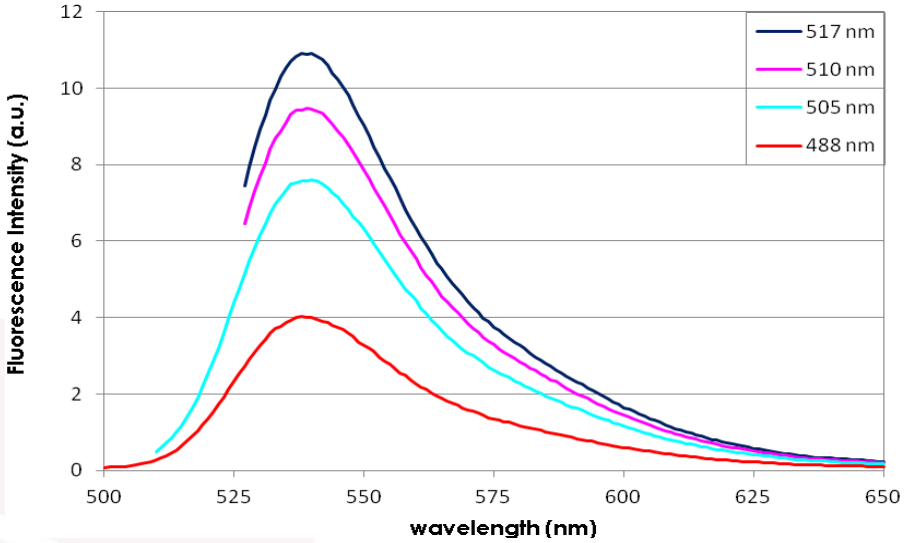
Effect of Excitation Wavelength on ANG-2 Emission Intensity



Emission spectra for a 150 mM NaCl solution of ANG-2 (TMA^+ Salt) in 130 mM TMACl, 10 mM MOPS, pH 7.1 at 488, 505, 510, and 517 nm excitation. Emission intensity drops to nearly a third of that at optimal (517 nm) excitation by exciting at 488 nm.

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ANG-2 (AM) Loading Protocol

A sample loading procedure may be accessed in Merck Research Laboratories' SLAS 2011 poster by O'Donnell, et al., for ANG-2 in a 1536-well voltage-gated sodium channel assay.

The following is supplied as a starting point for non-invasive loading of ANG-2 via the acetoxymethyl (AM) ester version. Due to cell type and other experimental variations, loading conditions will require optimization.

1. Prepare a 1 mM stock solution of ANG-2 (AM) in anhydrous dimethylsulfoxide (DMSO). *(A more concentrated solution may be used to minimize final DMSO percentage.)*
   - For a 500 µg vial, dissolve the contents of the vial in 460 µL DMSO.
   - For a 50 µg vial, use 46 µL DMSO.

2. Divide the stock solution into aliquots that will be consumed per experiment.
   - Store the aliquots at -20 °C, protected from moisture and light.
   - Repeated freezing and thawing of a stock solution typically leads to degradation of the product via hydrolysis of the AM esters
   - For your convenience, we supply ANG-2 (AM) in 50 µg packages.

3. Dilute the stock solution to twice the original volume with a solution of 20% Pluronic F-127 in DMSO.

4. Disperse the ANG-2/Pluronic F-127 solution into 100 times the volume of serum-free culture medium.
   - The final ANG-2 (AM) concentration is 5 µM. *(The concentration of ANG-2 AM may require optimization.)*
   - Pluronic F-127 is 0.1% of the final solution. *(The final concentration of Pluronic F-127 may require optimization.)*

5. Incubate the cells for one hour at room temperature. *(Incubation time and temperature, for example 37°C, may also require optimization.)*

6. Remove the cell loading medium and wash the cells with serum-free and dye-free medium.

7. The cells are now loaded with ANG-2 (AM) and ready for your sodium imaging experiments.

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Material Safety Data Sheet

Product Name	Asante NaTRIUM Green 2 (TMA^+ Salt), or ANG-2 (TMA^+ Salt)
Catalog Number	3522
Unit Size	250 µg/unit
Molecular Weight	1087 g/mol
Odor	None
Fire and Explosion Hazards	None
Handling and Storage	Store at -20°C. Protect from light and moisture
Toxicology Data	Not known
Emergency and First Aid	In case of contact with skin, eyes, or mouth, wash profusely with water. If swallowed, rinse mouth with water and seek medical advice.

For Further Information or in Emergency Contact the Manufacturer

Texas Fluorescence Labs, Inc
9415 Capitol View Drive
Austin, TX 78747
(512) 280-5223
probes@teflabs.com

Safety Precautions and Control Measures

Potentially harmful if inhaled or ingested. Do not get in eyes, on skin, or on clothing. Potential skin and eye irritant. Wash thoroughly after handling. Gloves should be worn when working with this material. Clean up procedure: wash with soap and water.

This material is for research and experimental applications only. It is not intended for food, drug, household, agricultural, or cosmetic use. All materials should be handled only by technically qualified individuals experienced in handling potentially hazardous chemicals. The above is correct to the best of our knowledge. The user should make independent decisions regarding completeness of the information based on all sources available. Texas Fluorescence Labs, Inc. shall not be held liable for any damage resulting from handling or from contact with the above product.

Revision 1.0, 03/08/2011

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Material Safety Data Sheet

Product Name	Asante NaTRIUM Green 2 (AM), or ANG-2 (AM)
Catalog Number	3502
Unit Size	500 µg/unit
Molecular Weight	1084 g/mol
Odor	None
Fire and Explosion Hazards	None
Handling and Storage	Store at -20°C. Protect from light and moisture
Toxicology Data	Not known
Emergency and First Aid	In case of contact with skin, eyes, or mouth, wash profusely with water. If swallowed, rinse mouth with water and seek medical advice.

For Further Information or in Emergency Contact the Manufacturer

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Revision 1.0, 03/15/2011

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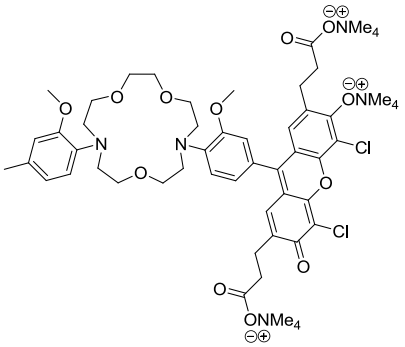
Product Specifications

Product Name	Asante NaTRIUM Green-2™ (TMA^+ Salt), or ANG-2 (TMA^+ Salt)
Catalog Number	3522 or 3552
Description	A non-ratiometric, green emission sodium indicator
Structure	
Molecular Weight	1087 g/mol
K_d for Na^+	20 mM (in the absence of K^+)
Solubility	Water
Handling and Storage	Store at -20° C. Protect from light and moisture
Shelf Life	Valid for one year after delivery, if stored properly

Specification

TLC	C_{18} silica
Solvent	7:3 methanol / 3M sodium acetate
R_f	0.5
HPLC	
Column	C_{18}
Detector Settings	254 nm and 475 nm
Purity	>90%
^{1}H NMR	All relevant peaks present
Solvent	Deuterated water
Absorbance Spectrum	
Solvent	Methanol
λ max	523 ± 3 nm
ε	85,000 $M^{-1}cm^{-1}$
Fluorescence Spectra	
Solvent	135 mM NaCl, 10 mM MOPS, pH 7.2
Emission maximum	540 ± 3 nm
Excitation maximum	517 ± 3 nm

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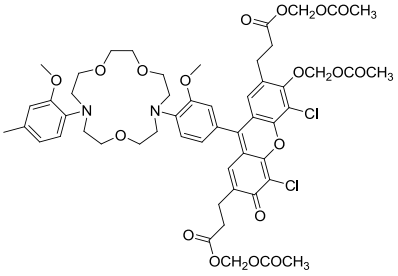
Product Specifications

Product Name	Asante NaTRIUM Green 2 (AM), or ANG-2 (AM)
Catalog Number	3502, 3512, 3532, or 3542
Description	Membrane permeable acetoxymethyl (AM) ester derivative of Asante NaTRIUM Green-2, a non-ratiometric green emission sodium indicator
Structure	
Molecular Weight	1084 g/mol
K_d for Na^+	20 mM (in the absence of K^+)
Solubility	DMSO
Handling and Storage	Store at -20° C. Protect from light and moisture
Shelf Life	Valid for one year after delivery, if stored properly

Specification

TLC

Solvent	5% methanol in chloroform
R_f	0.5

HPLC

Column	C_{18}
Detector Settings	254 nm, 460 nm
Purity	>90%

^{1}H NMR	All relevant peaks present
Solvent	Deuterated acetone

Absorbance Spectrum

Solvent	Methanol
λ max	469 ± 3 nm
ε	18,000 $M^{-1}cm^{-1}$

Fluorescence Spectra

Solvent	Methanol
Emission maximum	545 ± 3 nm
Excitation maximum	525 ± 3 nm

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