# ICR-1 AM

*05/21*

**ALTERNATE NAMES:** Calcium ($Ca2^+$) indicator; ICR-1 membrane permeable; ICR-1 acetoxymethyl ester; Fluorescent Calcium indicator

**CATALOG #:**
B3147-5PK 5 x 50 µg
B3147-500 500 µg

**MOLECULAR WEIGHT:** 1190.49

**APPEARANCE:** Colorless powder

**PURITY:** ≥ 90%

**SOLUBILITY:** Soluble in DMSO

**DESCRIPTION:** ICR-1 is a new red fluorescent calcium ($Ca^{2+}$) indicator for intracellular $Ca^{2+}$ measurements. It provides deeper tissue penetration and dramatic background reduction in tissues that have high autofluorescence, in the presence of serum or fluorescent compounds. ICR-1 can also be multiplexed with GFP-labeled cells or other green fluorophores. Unlike some other red fluorescent Ca2+ indicators, ICR-1 does not accumulate in the mitochondria. ICR-1 can be used for fluorescence lifetime imaging, multiphoton imaging and is ideal for most intracellular $Ca^{2+}$ measurements.

ICR-1 AM is the membrane permeable form of the calcium indicator. Masking the negative charge using non-polar, ester-linked moieties (AM ester) allows the molecule to enter cells through passive diffusion. Once inside the cell, ubiquitous intracellular esterase enzymes promote rapid hydrolysis of the AM ester leaving the active, highly polar form of the indicator trapped inside the cell. ICR-1 AM is optimal for cellular and tissue imaging applications due to the long-wavelength emission and a large Stokes shift which reduces contributions of autofluorescence. It binds to calcium with a $K_d$ value of 480 nM and displays excitation/emission maxima of 580 nm/660 nm, respectively.

**STORAGE TEMPERATURE:** -20 ºC. Store in the dark. Product is light sensitive. Protect from air. Store under desiccating conditions.

**HANDLING:** Do not take internally. Wear gloves and mask when handling the product! Avoid contact by all modes of exposure.

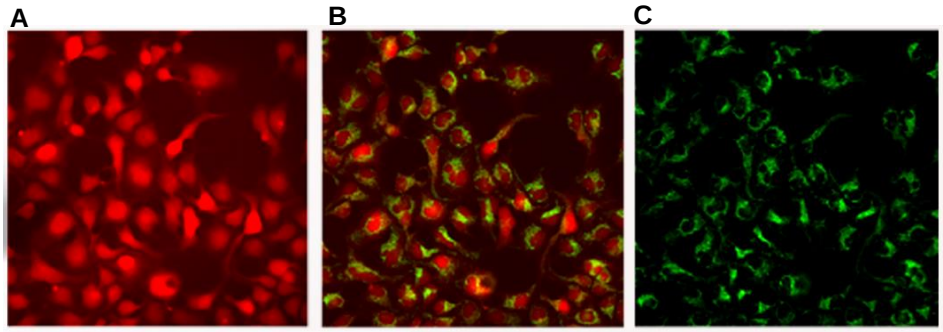
**PROTOCOL:**

User Supplied Reagents:
• DMSO
• Pluronic F-127 solution
• Probenecid solution (optional)
• HEPES-buffered Hank's Balanced Salt Solution
Procedure:

The following protocol provides general guidelines. All the loading conditions (concentrations, temperature and time) should be determined for specific assays, applications, and instrumentation.

1. Allow all reagents to warm to room temperature before proceeding.
2. Add 10 ml of assay buffer to a 15 ml conical tube. HEPES-buffered Hank's Balanced Salt Solution (pH 7.2-7.4) is the most commonly used assay buffer, although other buffers can also be used.
3. Add Pluronic F-127 solution to the assay buffer in conical tube. Pluronic F-127 is a biocompatible surfactant that aids in dye dissolution, ensuring equitable dye distribution and cellular loading.
4. Optional: Add Probenecid solution to the assay buffer. Probenecid is an anion transport inhibitor that improves intracellular dye retention. Although it is not required for all cell types and dyes, it is recommended in most cases to optimize assay performance.
   Note: Caution is advised when using probenecid as it may have undesirable effects on assay performance for the target of interest.
5. Vortex the conical tube briefly to mix.
6. Dissolve ICR-1 AM in 25 µl of DMSO. After adding DMSO, vortex tube briefly to dissolve the indicator dye, then centrifuge briefly to collect all contents at the tube bottom. Add entire contents of indicator dye tube to assay buffer solution to make a dye loading solution. The dye loading solution should be used within 2 h of dye addition for best results.
7. Vortex dye loading solution briefly to mix.
8. Remove the cell culture medium and add dye loading solution. Recommend volumes are: 35 mm dish or 6-well plate, 1.5 ml; 96-well plate, 100 µl; 384-well plate, 20 µl. When cells are sensitive to solution exchanges and will either be aspirated (suspension cells) or detach (loosely adherent cells) during wash steps, we recommend performing assays without removing any solution (no wash format) by adjusting the concentrations of reagents in the dye loading solution so that when everything is added to the wells, the final concentrations of all reagents are the same. If a no wash format is indicated for your application, we recommend doubling the concentration of all reagents in your dye loading buffer.
9. Incubate in a cell culture incubator at 37 ºC for 60 min.
10. Read the fluorescence using a plate reader (Excitation/Emission: 580 nm/660 nm) or image using a fluorescence microscope (using

filters for Texas Red).




Figures A, B and C show cells stained with ICR-1 and counterstained with mitochondrial stain in the red and green fluorescence channel respectively.

**RELATED PRODUCTS:**

Fluo-4 AM (Cat. No. B3146)
ING-2 TMA+ Salt (Cat. No. B3138)
IPG-2 AM (Cat. No. B3142)
IPG-1 AM (Cat. No. B3140)
FURA-5F/AM (Cat. No. 9551)

**DISCLAIMER:** ***FOR RESEARCH USE ONLY! Not to be used on humans.***