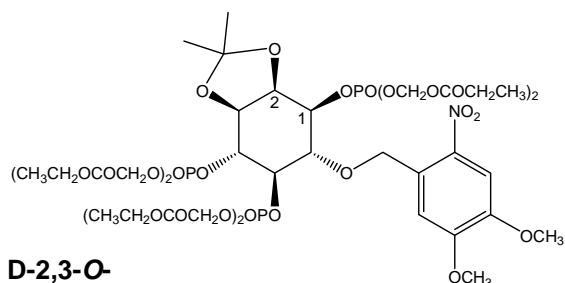


Photoactivatable and Membrane permeant Ins(1,4,5)P₃ (cag-iso-2-145)

D-2,3-O-Isopropylidene-6-O-(2-nitro-4,5-dimethoxy)benzyl-myoinositol 1,4,5-trisphosphate-Hexakis(propionoxymethyl) Ester

Product No.: cag-iso-2-145

Formula: C₄₂ H₆₄ N O₃₁ P₃

MW: 1171.27

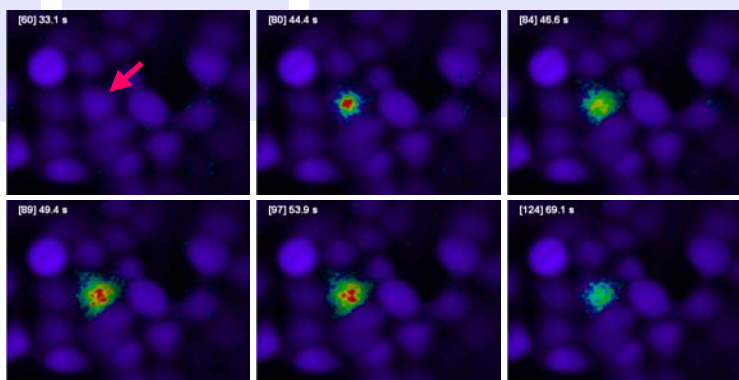
It is probably a good idea to aliquot the sample. The compound is soluble in dichloromethane, DMSO, which evaporates instantly under reduced pressure. The evaporation vessel should be filled with argon (better) or nitrogen afterwards. The compound is sensitive to water on a longer time scale. Therefore, please store the compound in substance or in dry DMSO (for not longer than 2 weeks) at -20°C or below. The freezing process should be performed very quickly (-80°C), not just in the freezer. For incubations, dissolve an aliquot of the cell penetrating compound in dry DMSO. Take out a small amount (e.g. 1 µl) and mix with same amount

of Pluronic F127 in DMSO (10%). To this mixture add 100 µl of the **serum-free cell supernatant**, mix thoroughly with a pipette and immediately add back to the cells. The final DMSO concentration in the experiment should not exceed 0.5%. The final concentration of caged-iso-Ins(1,4,5)P₃/PM should be in the **1-3 µM** range, depending on the cell type. For calcium measurements after de-caging use one of the standard calcium sensors. **Incubation** in the dark at room temperature for the cell penetrating compound and the calcium sensor should be **30-120 min**. Subsequently, it is possible to return to different buffers (with serum, if necessary). If buffer is not changed, **calcium levels** can be measured **within 5-10 min**. If buffers are changed, a longer adaptation phase (30 min) is recommended. To **un-cage** caged-iso-Ins(1,4,5)P₃/PM, scan cells once with an excitation around **360 nm** of an argon-ion UV laser or another UV light source

Summary:

- stock solution in CH₂Cl₂ or DMSO (storage: 2 weeks, -20°C)
- final concentration of cilnsP₃/PM: 1-3 µM
- incubation: 30-120 min
- uncage cilnsP₃/PM: with an argon-ion UV laser using the 345–355 nm line

In the shown experiment HeLa cells were co-loaded with 2 µM caged Ins(1,4,5)P₃/PM [cag-iso-2-145] and Oregon Green 488 BAPTA-1AM. All compounds were loaded for 80 min. at room temperature. Stimulus was 1 burst of 5 UV flashes (300-400 nm bandwidth) delivered 42 sec after the onset of the recording: [Ca²⁺]_i raised!



Experiments performed by Dr. Valeria Piazza and analysed by Dr. Catalin D. Ciobotaru in the laboratory of Prof. Fabio Mammano at the Venetian Institute of Molecular Medicine – Padua University, Italy, <http://www.vimm.it>