PRODUCT INFORMATION SHEET

Content and Storage

Product	Content	Storage	Stability
ChromaLive™ Fix Additive	Diluted in 10uL of DMSO	• 4°C • Delivered at room temperature • Protect from light	1 year

Intended Use
For research use only. Not for use in diagnostics or therapeutic procedures.

General Guidelines

Preceding steps (see ChromaLive protocol)

- Incubate cell with ChromaLive following standard protocols (see ChromaLive product datasheet)
- Incubate at least overnight to have stable and complete ChromaLive staining of live cells

Two modes of use are possible for ChromaLive Fix Additive ("the Additive")

- Mode 1 – Recommended if equipped with a microplate washer.
  Replace half of the existing volume with 2x preparation of the Additive. If equipped with a microplate washer, this allows to precisely leave half of the volume in each well, with minimal disturbance to cells
- Mode 2 – Recommended if NOT equipped with a microplate washer.
  Add 1/5 of the existing volume with 6x preparation of the Additive. This simplifies the assay and reduces possible variations in staining when not equipped with a microplate washer.

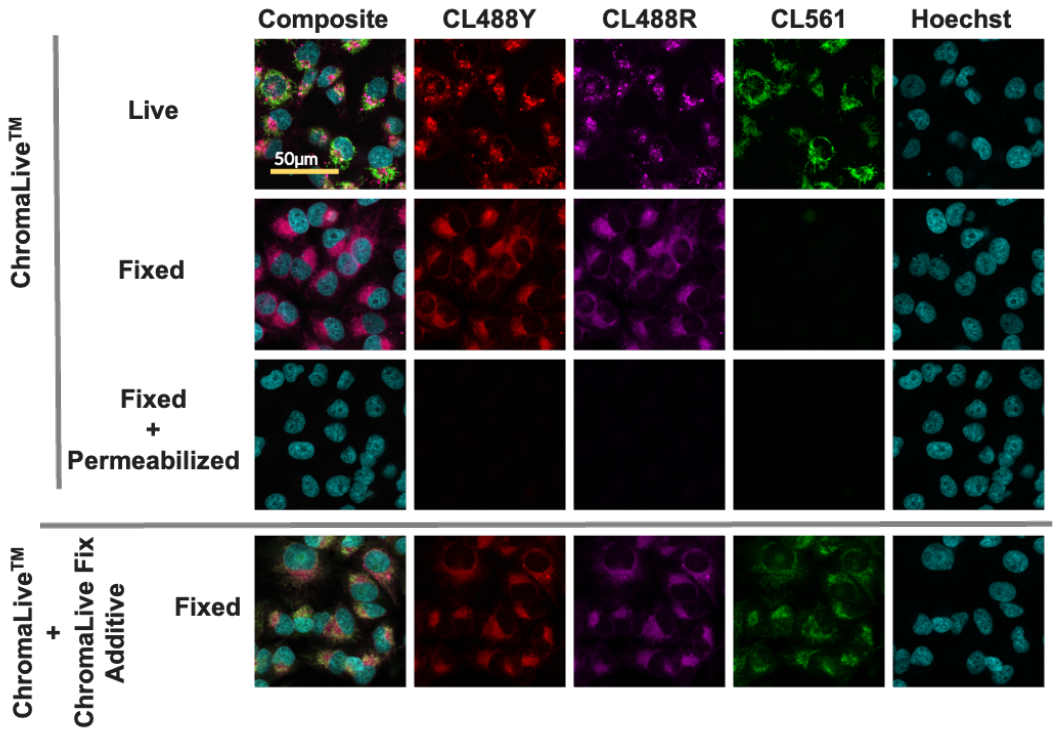

Figure 1. *MCF-7 cells imaged with ChromaLive in different conditions of fixation and in combination with ChromaLive Fix Additive. First row: live cell images. Second row: signal is partially lost after fixation without the Additive. Third row: signal from ChromaLive is completely lost after fixation and permeabilization, therefore making it compatible with downstream antibody staining. Fourth row: signal from ChromaLive is completely retained in presence of ChromaLive Fix Additive. See ChromaLive Protocol for general acquisition settings.*

Mode 1 – Replacing half of the well volume with 2x Additive

- Prepare ChromaLive Fix Additive (2x)
  - Dilute the Additive 1:500 in preferred culture medium (e.g., dilute 10µL of Additive in 5mL complete RPMI)
- Remove half of the well volume (e.g., for 384 well plates with 50µL / well, remove medium to leave 25µL in each well)
- Add this same volume of 2x Additive to each well (continuing with the previous example: add 25 µL of 2x Additive), bringing the final Additive concentration to 1x.
- Incubate at 37°C for 2h.
- Fix cells with 4% PFA for 30 minutes at room temperature, protected from light
  - To minimize cell perturbation, it is recommended to add a smaller volume of concentrated PFA (e.g., to a well containing 50µL, add 12.5µL of PFA 20%, or 16.7µL of PFA 16%)
- Wash plate 4 times with warm PBS, leave a final volume of PBS in each well
- Image plate of fixed cells, or store at 4°C (for up to 2 weeks)

Mode 2 – Adding volume of 6x Additive

- Prepare ChromaLive Fix Additive (6x)
    - Dilute the Additive 3:500 in preferred culture medium (e.g., dilute 6µL of Additive in 1mL complete RPMI)
- Add 1/5 of the well volume of 6x Additive (e.g., for 384 well plates with 50µL / well, add 10µL of 6x Additive), bringing the final Additive concentration to 1x.
- Incubate at 37°C for 2h.
- Fix cells with 4% PFA for 30 minutes at room temperature, protected from light
    - To minimize cell perturbation, it is recommended to add a smaller volume of concentrated PFA (e.g., to a well containing 60µL, add 15µL of PFA 20%, or 20µL of PFA 16%)
- Wash plate 4 times with warm PBS, leave a final volume of PBS in each well
- Image plate of fixed cells, or store at 4°C (for up to 2 weeks)