PRODUCT INFORMATION SHEET

Content and Storage

Product	Content	Storage	Stability
ChromaLive™ Non-Toxic Dye	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

ChromaLive dye preparation and dilution:

- Warm up the ChromaLive dye tube to room temperature before use to avoid condensation to form and water to get into the anhydrous dye solution
- Gently spin the tube before use to collect any dye solution that may remain near the cap
- Dilute 10µL ChromaLive dye in 10mL of RPMI complete medium (1:1000 the provided solution). Vortex thoroughly and keep on ice.

Cell culture protocol and compound testing, with ChromaLive dye

- Seed cells at desired density on cell culture dish, in previously prepared culture medium with ChromaLive dye
- Add control compounds, test compounds and negative controls for phenotypes of interest.
- NOTE: ChromaLive dye can also be added after cell seeding and compound addition. In that case, incubate dye for at least 3h at 37°C, 5% CO_2 before imaging.

Imaging

- NOTE: Keep ChromaLive dye in solution while imaging, no need for a washing step.
- NOTE: Nuclear staining can be added for cell segmentation during image analysis. Check manufacturer's guidelines.
- Imaging conditions: the supplied ChromaLive dye needs to be imaged at 3 different wavelengths which can be identified in the following manners:
    - ChromaLive488_Yellow: excitation at 488nm, image acquisition between 550-630nm
    - ChromaLive488_Red: excitation at 488nm, image acquisition between 630-750nm
    - ChromaLive561: excitation at 561nm, image acquisition between 590-630nm
- NOTE: more information regarding imaging and filter sets is available at info@saguarobio.com, where our team can check set-up compatibility.

Standard Protocol for 3D Cultures

Some considerations

- Material, reagents and volumes used herein are suitable for 3D culture of MCF-7 cells in 384-well plate and need to be adjusted according to the experimental design.
- Complete culture medium composition: RPMI 1640 (ATCC modification), supplemented with 10% FBS and 1% Penicillin/Streptomycin
- Thaw *Matrigel* overnight at a 4°C before use.
- Keep Matrigel on ice during the entire process.

ChromaLive dye preparation and dilution (day0):

- Warm up the ChromaLive dye tube to room temperature before use to avoid condensation to form and water to get into the anhydrous dye solution
- Gently spin the tube before use to collect any dye solution that may remain near the cap
- Dilute 10µL ChromaLive dye in 10mL of RPMI complete medium (1:1000 the provided solution). Vortex thoroughly and keep on ice.

Cell culture protocol with ChromaLive dye (day0):

- Coat the desired number of wells of a pre-chilled 384-well black walled plate with 10µL of Matrigel,
- Spread Matrigel evenly with a pipet tip, then incubate at 37°C for 30 min to allow for polymerization of the Matrigel matrix.
- Wash the MCF-7 cells once with PBS. Harvest the cells from the culture flask using the routine trypsinization procedure, then centrifuge at 125 x g for 5 min at room temperature (RT).
- Add Matrigel to 5% of the final volume of the previously prepared ChromaLive containing RPMI complete medium *(0.5 mL Matrigel + 9.5 mL medium)*. Mix well using a pipet or vortex.

- Re-suspend the cells with the ChromaLive/Matrigel containing RPMI medium at a final cell density of 125 x 10^3 cells/mL (5000 cells/well). Homogenize the cell suspension by Up & down, using a 5mL serological pipet.
- Plate 40µL cell suspension into each well of the pre-coated wells and place the multi-well plate in the cell incubator (37%, 5% CO_2, 95% Air).
- Replace medium every two days.

(OPTIONAL) Hoechst labeling of nucleus, for cell segmentation (day1)

- Add Hoechst 33342 solution at 1µg/mL in culture medium, add 5µL per well for a final concentration of 100ng/mL. Incubate for at least 3h, at 37°C, 5% CO_2, before imaging.

Compound preparation and testing (day3)

- Prepare test compounds of interest at the desired concentrations (1:10 serial dilutions), while maintaining constant vehicle solvent concentration. As a reference, Thapsigargin compound could be prepared at 10nM as the highest concentration.
- Prepare negative controls with vehicle solvent (here, 0.1% DMSO)
- Add test compounds or controls to the corresponding wells.

Imaging and data acquisition (day3-5)

- Image 384 well plate at the desired timepoints, using a 20X objective and suitable filter settings (See *Additional information*).

Materials:

- Cell line: MCF-7 (ATCC, ref: HTB-22)
- 384-well plate: PhenoPlate 384-well, black, optically clear flat-bottom (PerkinElmer, ref: 6057328)
- Culture medium: RPMI 1640 (ATCC modified) (Gibco, ref: A1049101)
- Trypsin: 0.25% Trypsin/EDTA (Corning, 25-053-CI)
- Serum: FBS (Corning, ref 35-015-CV)
- BME: Phenol-free GFR Matrigel (Corning, ref: 356231)
- Nuclear stain: Hoechst 33342 (Invitrogen, ref: H1399)
- Vehicle: DMSO (Fisher Scientific, ref: BP231-100)
- Buffer: PBS (Corning, ref: 21-040-CV)

Additional Information

Acquisition channels and examples of filter settings

- ChromaLive488_Yellow
  - Excitation: 488nm laser OR 475/34nm excitation filter
  - Acquisition: 593/40nm emission filter
- ChromaLive488_Red
  - Excitation: 488nm laser OR 475/34nm excitation filter
  - Acquisition: 692/40nm emission filter
- ChromaLive561
  - Excitation: 561 laser OR 560/32nm excitation filter
  - Acquisition: 593/40nm emission filter
- Optional: DAPI
  - Excitation: 405nm laser OR 377/54nm excitation filter
  - Acquisition: 447/60nm emission filter

Image Examples

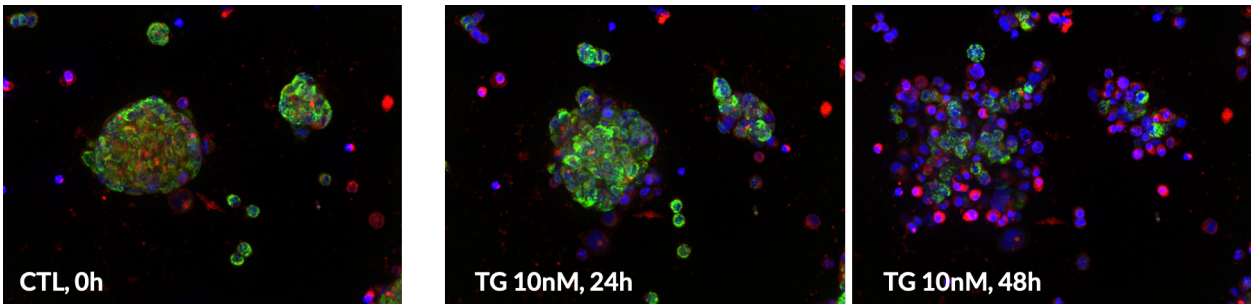

Figure 1. 3D culture of *MCF-7 cells with ChromaLive*. *Left is the control condition, and the two right images are cells treated with Thapsigargin – an ER stress inducer – respectively 24h and 48h after treatment. Red: CL-488 Yellow, Green: CL-561 Yellow, Blue: Hoechst 33342. The CL-488 Red channel is omitted for these example images, although it was acquired and is recommended for image analysis. At 24h, decrease in CL-488 Yellow and increase in CL-561 Yellow signals are observed. At 48h, increase in CL-488 Yellow around the nucleus and decrease in CL-561 Yellow signals are observed.*

Control Compound Examples

Doses and duration for MCF-7 cells in 2D

Cell death mechanism	Control compounds (Concentration range, 1:10 serial dilution)	End-point	Time points
Apoptosis	Actinomycin D (1pM-1µM) Staurosporine (5pM-5 µM)	Actinomycin D: 72h Staurosporine: 24h	12h, 24h, 48h, 72h 3h, 6h, 12h, 24h***
ER stress	Tunicamycin (10pM-10µM) Thapsigargin (1pM-1µM)	Tunicamycin: 24h Thapsigargin: 24h	3h, 6h, 12h, 24h 3h, 6h, 12h, 24h***
Autophagy	Rapamycin (10pM-10µM)	Rapamycin: 72h	12h, 24h, 48h, 72h

*Only provided as examples. Controls require validation.
**Images could be collected more frequently with the appropriate equipment, especially for time-lapse imaging (controlled temperature and CO_2, auto-focusing, etc.)
***See ChromaLive white paper