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CellVue® Maroon Membrane Staining Protocol
For Isolating, Tracking and Characterizing Somatic or Cancer Stem Cells and their Lineages

Overview:

Spheres cultures are a model for studying the properties of somatic stem cells (SSCs) or cancer stem cells (CSCs) and their cell lineages [1, 2, 3]. CellVue Maroon fluorescent dye for live cell staining has ideal properties for isolating, tracking and characterizing SSCs or CSCs and stem cell derived cell lineages. CellVue Maroon is very stable, non-cytotoxic and compatible with commonly used Green Fluorescent Protein (GFP) or FITC (Fluorescein IsoThioCyanate) fluorochrome cell detection. As a membrane labeling dye, dilution or retention of CellVue Maroon is a measure of the rate of proliferation of a cell and its descendant cells. Maroon analysis and Fluorescence Activated Cell Cytometry (FACS) profiling during SSC self-renewal and differentiation provides insight into cell division cycles of specific cell types generated by SSCs. As SSCs often retain low proliferation in vivo and in sphere formation assays, SSCs display the highest Maroon cell dye retention with Maroon labeling [4, 5].

Protocol for CellVue® Maroon Staining (adapted from Piscitelli et al, Reference 5).

While the protocol is shown for mammospheres generated from single stem cells derived from dissociated human breast tissue, the protocol is suitable for cell lines, and for cells from other tissues and from other species.
Mammospheres (that contain self-renewing stem cells) are collected with a 40 µm cell- strainer with single cells or small cell aggregates removed with the wash through. The spheres are then retrieved from the cell-strainer and enzymatically dissociated with 1.25% Trypsin-EDTA (approximately 5 to 25 minutes) to obtain single cells for Maroon labeling.

Labeling is performed in a 2 µM final concentration of CellVue Maroon.

1. Count the single cells (in PBS), centrifuge (400 rcf) and re-suspend in Diluent C at a concentration of 2 X 10^3 cells/µl (resulting in a 2X cell concentration) in a 15ml tube.
2. In a different 15ml tube prepare a 2X working dye stock (4 µM Maroon) in Diluent C and mix well by pipetting.
3. Mix rapidly together the 2X cell suspension and the 2X working dye stock and incubate for 5 minutes at room temperature.
4. Stop the reaction by adding an equal volume of 100% Fetal Bovine Serum and incubate for 1 minute.
5. Perform two washes with 10 ml of FBS containing medium.
6. Re-suspend the cells in the appropriate tissue culture media and plate accordingly in suspension conditions appropriate for generating secondary spheres (for instance a limiting cell dilution condition is equal to or less then 1000 cells per 2.5ml of tissue culture media).

Maroon staining of spheres can be visualized by fluorescence microscopy with a Cyanine 5 (Cy5) filter (Figure 1). Cells with different Maroon intensities (a measure of dye dilution with cell division) can be isolated with Fluorescence Activated Cell Sorting (FACS) (Figure 2).

Figure 1 displays a typical Maroon dye dilution profile of a 7 day old sphere generated from a single mammary gland stem cell derived from a human breast tissue of a healthy patient.
Figure 2 is a typical FACS profile obtained from single cells of dissociated mammospheres generated from Maroon labeled mammary gland stem cells.

Fig 1

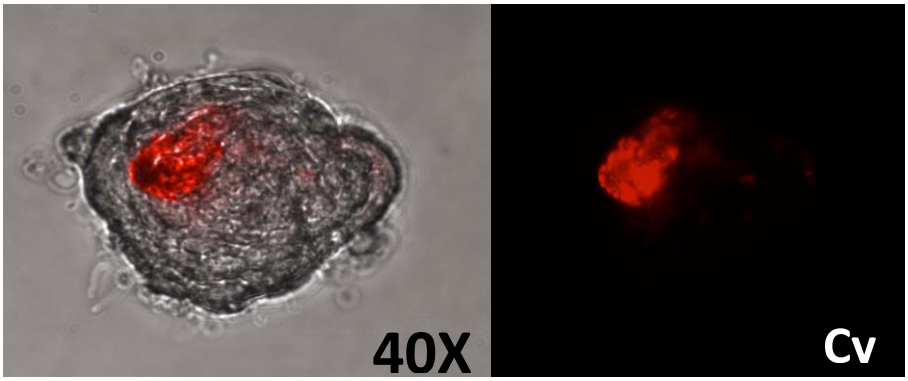
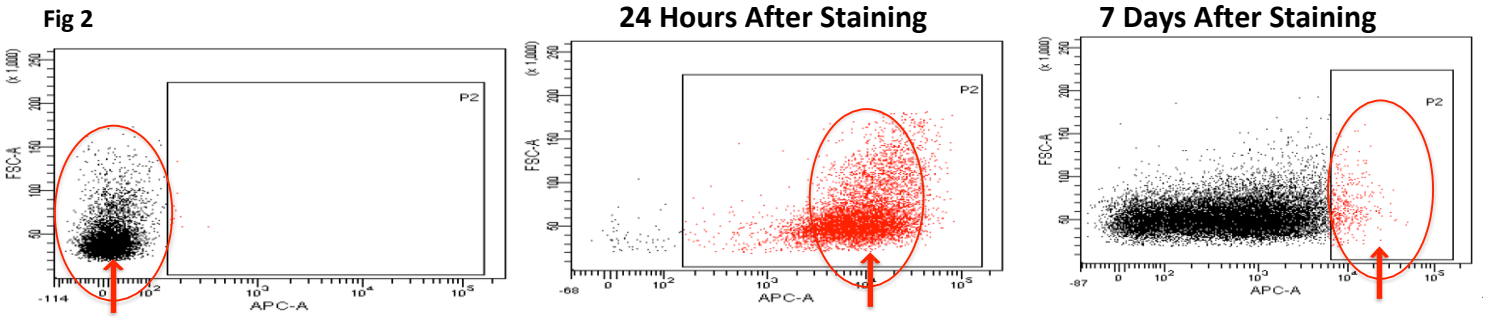


Figure 1. Fluorescence microscopy image of a mammosphere generated from a single Maroon dye stained human mammary gland stem cell from a healthy patient. Mammosphere cells with highest Maroon intensity have stem cell *features* *(see Reference 5). Image property of Eleonora Piscitelli and Istituto di Tecnologie Biomediche Consiglio Nazionale delle Ricerche, Italy.*

Fig 2



Unstained Mammosphere cells

Homogeneous Maroon staining (APC) of mammosphere cells (red)

The population of mammosphere cells with highest Maroon staining (APC) is enriched with mammary gland stem cells (red)

Figure 2. FACS profile obtained from single cells of dissociated mammospheres generated from Maroon labeled mammary gland stem cells. (see Reference 5). Image property of Eleonora Piscitelli and Istituto di Tecnologie Biomediche Consiglio Nazionale delle Ricerche, Italy.

Protocol notes:

1. While not less then 50,000 cells in a final volume of 50 microliters should be used to obtain consistently homogenous Maroon staining, staining can also be performed with much less cells or even a single cell.
2. Cells should not remain in Diluent C for more than 3 - 5 minutes before Step 3 as this results in decreased cell viability.
3. It is critical that the cell suspension and the dye solution are mixed as rapidly as possible to achieve homogeneous cell staining.
4. All Maroon containing steps are performed with as little light as possible to minimize dye bleaching.
5. Immediately after Maroon dye staining of cells, fluorescence with the Cyanine 5 (Cy5) filter is not observed with microscopy. Fluorescence detection with microscopy is possible only after 2 to 3 hours of cell labeling.

References:

1. Dontu G, Wicha MS (2005). Survival of mammary stem cells in suspension culture: implications for stem cell biology and neoplasia. **J Mammary Gland Biol Neoplasia** 10:75–85.
2. Zucchi I, Sanzone S, Astigiano S et al (2007) The properties of a mammary gland cancer stem cell. **Proc Natl Acad Sci USA** 104:10476–10481.
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4. Pece S, Tosoni D, Confalonieri S et al (2010) Biological and molecular heterogeneity of breast cancers correlates with their cancer stem cell content. **Cell** 140:62–73.
5. Piscitelli E, Cocola C, Thaden FR, et al (2015). Stem Cell Protocols, Methods in Molecular Biology, Vol 1235, DOI 10.1007/978-1-4939-1785-3-18, Copyright Springer Sciences & Business Media New York 2015.