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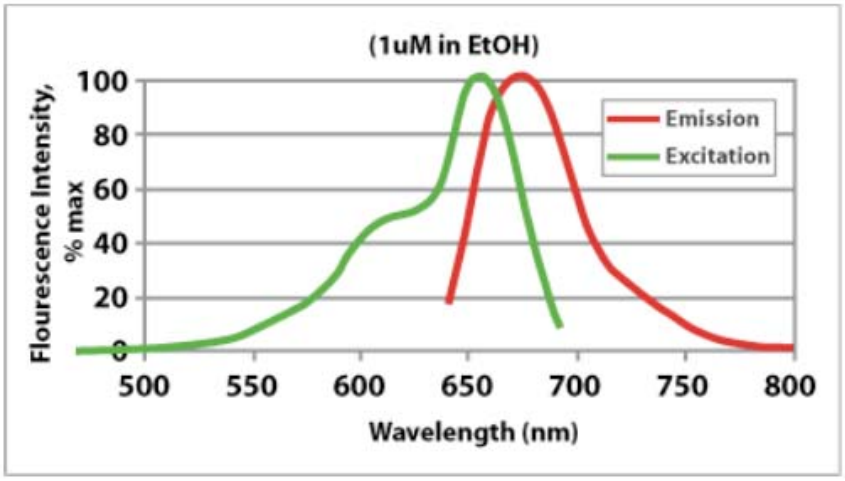
Catalog Number: C-1002

Product Name: CellVue® Claret Fluorescent Cell Linker Kit For General Cell Labeling

Product Description:
The CellVue Claret cell linker kit uses proprietary membrane labeling technology to stably incorporate a fluorescent dye with long aliphatic tails (CellVue Claret) into lipid regions of the cell membrane (1).The labeling vehicle provided with the kit (Diluent C) is an iso-osmotic aqueous solution which contains no physiologic salts or buffers, detergents, or organic solvents and is designed to maintain cell viability while maximizing dye solubility and staining efficiency. The pattern of staining is dependent upon the cell type being labeled and the membranes of the cells (2, 3). CellVue Claret, a far red fluorescent cell linker (Figure 1) has been reported to be useful for *in vitro* cell labeling (4*)* and *in vitro* T-cell proliferation studies (4).

Figure 1. CellVue Claret Excitation and Emission Spectra.(ex. max = 655nm; em max= 675nm)



Kit Components :

Mini Size

- CellVue Claret dye stock (1 vial containing 0.1 ml, 1 x 10^{-3} M in ethanol)
- Diluent C (1 vial containing 10 ml)

Midi Size

- CellVue Claret dye stock (2 vials each containing 0.1 ml, 1 x 10^{-3} M in ethanol)
- Diluent C (6 vials each containing 10 ml)

Storage/Stability

- CellVue Claret dye stock in ethanol may be stored at room temperature or refrigerated.
- To prevent increase in dye concentration due to evaporation, **the dye stock in ethanol should be kept tightly capped** except when in immediate use.
- Dye stock must be protected from bright direct light and examined for crystals prior to use. If crystals are noted in the dye stock, it should be warmed slightly in a 37°C water bath and sonicated or vortexed to redissolve the crystals.
- Diluent C may be stored at room temperature or refrigerated. If refrigerated, bring to room temperature before it is used to prepare cell and dye suspensions used for labeling (steps 5 and 6 under General Cell Membrane Labeling, below).

- Diluent C is provided as a **sterile** solution. Because it does not contain any preservatives or antibiotics, it should be kept sterile.
- Working solutions of dye in Diluent C should be made **immediately** prior to use.
- Do not store dye in Diluent C.

Procedures

Materials Required for General Membrane Labeling but not included:

- A uniform suspension of single cells in tissue culture medium
- Tissue culture medium with serum
- Ca^{2+}, Mg^{2+} and serum free medium or buffered salt solution (e.g. Dulbecco's PBS or Hank's BSS)
- Serum, albumin or other system-compatible protein source
- Polypropylene conical centrifuge tubes
- Temperature-controlled centrifuge (0 to 1,000 x *g*)
- Instrument(s) for analysis of fluorescence (fluorometer, fluorescence microscope, flow cytometer, or fluorescence image analysis instrumentation)
- Laminar flow hood
- Hemocytometer or cell counter
- Slides and coverslips

General Cell Membrane Labeling

The appearance of labeled cells may vary from bright and uniform labeling to a punctate or patchy appearance depending on cell type. **Labeling occurs by partitioning of the lipophilic dye into cell membranes and labeling intensity is a function of both dye and cell concentration. Labeling is not saturable. Therefore, it is essential that the amount of dye available for incorporation be limited**. Over-labeling of the cells will result in loss of membrane integrity and reduced cell recovery. The cell and dye concentrations given in the following example represent starting concentrations that have been found broadly applicable to a variety of cell types. **To maximize fluorescence per cell while maintaining viability and functionality, users must determine the optimum dye and cell concentrations for their cell type(s) and experimental purposes. Also, the user should evaluate cell viability (e.g., propidium iodide exclusion), fluorescence intensity, coefficient of variation of fluorescence peaks, and uniformity of staining** (5)**.**

To stain at final concentrations of 2×10^{-6} M CellVue Claret dye and 1×10^{7} cells/mL in a final staining volume of 2 mL perform the following using aseptic techniques:

1. Adherent or bound cells must first be removed from the culture vessel using proteolytic enzymes (i.e., trypsin/EDTA) and put into a single cell suspension.

Perform all subsequent steps at ambient temperature (20 -25 °C.).

2. Place a total of approximately 2×10^{7} single cells in a conical bottom polypropylene tube and wash once using medium without serum.
3. Centrifuge the cells (400 x *g*) for 5 minutes into a loose pellet.
4. After centrifuging cells, carefully aspirate the supernatant, being careful not to remove any cells but leaving no more than 25 µLof supernatant on the pellet.
5. Prepare 2x cell suspension by adding 1 mL of Diluent C to cell pellet and resuspending with gentle pipetting to insure complete dispersion. Do not vortex.
6. **Immediately** prior to staining, prepare 2x working dye stock (4×10^{-6} M CellVue dye) in Diluent C (supplied with the kit). To 1 mL of Diluent C in a polypropylene centrifuge tube add 4µL of 1×10^{-3} M dye stock in ethanol. To minimize ethanol effects on cells, the amount of dye added should be less than 1% of final staining volume at the end of Step 7. If a greater dilution of the dye stock is necessary, make an intermediate stock by diluting with 100% ethanol.
7. Rapidly add the 1 ml of 2x cell suspension from Step 5 to 1 ml of 2x working dye from Step 6, and **immediately** mix the sample by pipetting. **Note: rapid and homogeneous mixing is critical for uniform labeling because staining is nearly instantaneous**.
8. Incubate the cell/dye suspension from Step 7 (1×10^{7} cells/mL, 2×10^{-6} M dye) for 2 to 5 minutes with periodic mixing. Longer staining periods will result in brighter cell staining but should be checked for effects on cell viability and function.
9. Stop the staining by adding an equal volume (2 mL) of serum or compatible protein solution (i.e., 1% BSA). Incubate 1 min. Do not dilute with Diluent C.
10. Centrifuge the cells at 400 x *g* for 10 minutes at 25 °C to remove cells from staining solution. Carefully remove

supernatant, being sure not to remove cells.

11. Wash the cell pellet 3 times with 10 mL of complete medium to ensure removal of unbound dye. **Note**: to minimize effects of residual dye bound to tube walls, transfer cell/dye suspension to a fresh, sterile conical polypropylene tube at the first resuspension step. Do not wash with Diluent C.
12. After final wash, resuspend cell pellet in 10 mL complete medium for cell counting and viability determination. After assessing cellular recovery and viability, centrifuge and resuspend to desired concentration of viable cells.
13. Examine the cells using fluorescence microscopy, flow cytometry or fluorescence-based image analysis. The stained sample should be checked for cell recovery, cell viability, fluorescence intensity and function. Staining is typically 100-1,000 times brighter than background autofluorescence. Distributions should be symmetrical and as homogeneous as possible, although staining CV will depend on the cell type being stained.

Critical Aspects of General Cell Membrane Labeling

14. No azide or metabolic poisons should be present at the time of CellVue Dye staining.
15. It is imperative that single cell suspensions be used, to obtain uniform staining.
16. Prior to staining, remove all serum proteins and lipids that may reduce the effective dye concentration available for labeling.
17. The presence of physiologic salts causes the dye to form micelles and substantially reduces staining efficiency. Therefore, it is important that the cells be resuspended in Diluent C prior to the addition of dye, not medium or buffered salt solution. It is also important that the amount of residual supernatant remaining prior to resuspension in Diluent C be minimized (Step 4) .
18. Ethanolic dye stock should not be added directly into the cell suspension. This will result in poor mixing and heterogeneous staining distributions
19. Ethanolic dye stock should not be added directly to the cell pellet. This will result in poor cell viabilities.
20. Rapid and homogeneous mixing is critical for uniform labeling. The following measures have been found to aid in achieving optimum results:
    a) Mix equal volumes of cell suspension and working dye solution;
    b) Avoid staining in very small (<100 µL) or very large (>5 mL) volumes.
    c) Avoid the use of serological pipettes for the addition of cells to dye.
    d) Dispense volumes as precisely as possible so that both cell and dye concentrations are accurately reproduced from sample to sample and study to study.
21. To minimize loss of viability, expose cells to dye and diluent for the minimum time needed to achieve the desired staining intensity. Since Diluent C lacks physiologic salts, longer exposure may cause reduced viability in some cell types. To test for diluent effects, run a mock-stained control in which ethanol rather than dye is used in Step 6 of the staining procedure and test for viability or functional properties of interest.
22. Do not centrifuge the cells in Diluent C before stopping the staining reaction.
23. Staining should be stopped by adding an equal volume of serum or other suitable protein source. Serum is preferred.
24. Washing efficiency is increased if serum proteins or albumin are added to the stop and washing solutions. It is also increased by transfer into a fresh tube at the first resuspension step after staining is stopped (Step 11).
25. Do not use Diluent C for washing steps.
26. This labeling procedure can be used for *in vitro* or *ex vivo* labeling of stem cells, lymphocytes, monocytes, endothelial cells or any other cell type where partitioning of dye into lipid regions of the cell membrane is desired. Alternate methods may be more suitable for *in vivo* labeling and/or selective labeling of phagocytic cells (6-8).
27. General cell labeling should be performed prior to monoclonal antibody staining. The cell tracking probes will remain stable during the monoclonal staining at 4 °C; however, capping of the monoclonal antibodies is highly probable if the general cell labeling is carried out at ambient temperature subsequent to antibody labeling.
28. Stained cells may be fixed with 2% methanol-free paraformaldehyde and intensities are expected to be stable for several weeks so long as the samples are protected from direct bright light.
29. Platelet (or liposome) labeling requires a modification of this protocol (2).

Histology

Preparation and preservation of slides containing cells labeled with lipophilic membrane dyes requires frozen tissue sectioning and special mounting techniques as such dyes are partially or completely soluble in many organic solvents. The following methods, developed by Drs. Per Basse and Ronald H. Goldfarb (Pittsburgh Cancer Institute, Pittsburgh, PA) for use with visible fluorescent PKH family of membrane dyes, are also expected to be useful for tissues containing cells labeled with CellVue dyes.

Preparation of slides

1. Excise tissues to be sectioned and freeze immediately on dry ice.
2. Store tissues at –70 °C prior to sectioning.
3. Mount frozen tissues using O.C.T. compound (Tissue-Tek; Miles, Inc.)
4. Prepare 4 to 5 micron tissue sections.
5. Air dry slides for at least 1 hr at room temperature.
6. Mount coverslip using 1-2 drops of cyanoacrylate ester glue. (Successful results have been obtained using the following brands of cyanoacrylate ester glue: Elmer's Wonder Bond, Archer Instant Bonding Adhesive, Bondo Super Glue, Duro Super Glue, Scotch Instant Glue and Instant Krazy Glue).
7. Examine or photograph sections using standard filter setup for Cy5 (CellVue Claret).

Counterstain Sections

1. Remove coverslips by soaking slides in acetone for 24 to 48 hours.
2. Rinse slides in distilled water to remove acetone.
3. Counterstain sections using stain of choice. Satisfactory results have been obtained using Mayer's or Harris hematoxylin.
4. Mount slides using AS/AP permanent aqueous mounting medium (Bio/Can America, Inc.,Portland, ME).

Note: Because organic solvents may extract CellVue dyes and counterstains may absorb fluorescence, simultaneous visualization of CellVue fluorescent cell linker dyes and histological staining has not been demonstrated. Use serial sections or use a single section and perform fluorescent microscopy before demounting and counterstaining (5). For a protocol successfully used to detect PKH26, a visible-emitting membrane dye, in combination with an immunohistochemical counterstain, see Soukiasian *et al* (9).

References

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This product is offered for research purposes only and is not intended for human therapeutic or diagnostic use.

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Version 5: Jan 2009