

CellVue® Kits for Membrane Labeling

- Versatile – use with any cell type or bioparticle with a membrane
- Provide stable labeling with minimal transfer from cell to cell
- Provide rapid, uniform membrane labeling
- Able to combine with fluorescent antibodies or markers of cell function
- Suitable for Cell Tracking and Proliferation studies
- Several colors (UV to NIR) for multi-parameter studies (use with existing fluors for more colors)
- Far-Red and NIR versions can provide greater S/N due to reduced background autofluorescence
- Compatible with flow cytometers, confocal and in vivo imaging equipment
- Convenient, easy-to-use kit format

Product Description

The CellVue® cell linker kit uses proprietary membrane labeling technology to stably incorporate a fluorescent dye with long aliphatic tails (CellVue® Dye) 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).

Product Name	Ex. Max / Em. Max	Description
CellVue® Burgundy	Ex max=683nm Em max = 707nm	Far red/near infrared fluorescent cell linker that has been reported to be useful for cell tracking in isolated organ preparations applications (4).
CellVue® Claret	Ex max=655nm Em max = 675nm	Far red fluorescent cell linker, that has been reported to be useful for <i>in vitro</i> cell labeling (5) and <i>in vitro</i> T-cell proliferation studies (5).
CellVue® Jade	Ex max=478nm Em max = 508nm	Green fluorescent cell linker, useful for cell tracking applications.
CellVue® Lavender	Ex max=420nm Em max = 461nm	Long wavelength UV fluorescent cell linker that has been reported to be useful for <i>in vitro</i> cell labeling (6) and <i>in vitro</i> cell proliferation studies (6).
CellVue® Maroon	Ex max=647nm Em max = 667nm	Far red fluorescent cell linker that has been reported to be useful for <i>in vitro</i> cell tracking (7), <i>in vitro</i> cell proliferation monitoring (7), cell tracking in isolated organ preparations applications (8) and studying membrane transfers between immune cells (9).
CellVue® NIR780	Abs max=743nm Em max = 776nm	Near infrared fluorescent cell linker that has been reported to be useful for <i>in vitro</i> cell proliferation monitoring (6).
CellVue® NIR815	Ex max=786nm Em max = 814nm	Near infrared fluorescent cell linker that has been reported to be useful for short term <i>in vitro</i> cell proliferation studies (10), cell tracking in isolated organ preparations applications (8) and cell tracking studies using the Odyssey Near Infrared Imaging System (Li-COR Biosciences, Inc. Lincoln, NE) (11).
CellVue® Plum	Ex max=652nm Em max = 671nm	Far red fluorescent cell linker that has been reported to be useful for <i>in vitro</i> cell tracking (6) and <i>in vitro</i> cell proliferation monitoring (6).

Kit Sizes & Components

Mini Size

- CellVue® 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® dye stock (2 vials containing 0.1 ml, 1 X 10⁻³ M in ethanol)
- Diluent C (6 vials containing 10 ml)

Should any of our materials fail to perform to our specifications, we will be pleased to provide replacements or return the purchase price. We solicit your inquiries concerning all needs for life sciences work. The information given in this bulletin is to the best of our knowledge accurate, but no warranty is expressed or implied. It is the user's responsibility to determine the suitability for his own use of the products described herein, and since conditions of use are beyond our control, we disclaim all liability with respect to the use of any material supplied by us. Nothing contained herein shall be construed as a recommendation to use any product or to practice any process in violation of any law or any government regulation

Materials Required for General Membrane Labeling but not included:

- A uniform suspension of single cells in tissue culture medium
- Tissue culture medium with serum
- Ca²⁺, Mg²⁺ and serum free medium or buffered salt solution
- 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 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 (12).**

To stain at final concentrations of 2×10^{-6} M CellVue[®] 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 μ L of 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 4 μ L 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.
11. Carefully remove supernatant, being sure not to remove cells, and 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. Intensity distributions should be symmetrical and as homogeneous as possible, although staining CV will depend on the cell type being stained.

Should any of our materials fail to perform to our specifications, we will be pleased to provide replacements or return the purchase price. We solicit your inquiries concerning all needs for life sciences work. The information given in this bulletin is to the best of our knowledge accurate, but no warranty is expressed or implied. It is the user's responsibility to determine the suitability for his own use of the products described herein, and since conditions of use are beyond our control, we disclaim all liability with respect to the use of any material supplied by us. Nothing contained herein shall be construed as a recommendation to use any product or to practice any process in violation of any law or any government regulation

Critical Aspects of General Cell Membrane Labeling

1. No azide or metabolic poisons should be present at the time of CellVue® Dye staining.
2. It is imperative that single cell suspensions be used, to obtain uniform staining.
3. Prior to staining, remove all serum proteins and lipids that may reduce the effective dye concentration available for labeling.
4. 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, of General Labeling).
5. Ethanolic dye stock should not be added directly into the cell suspension. This will result in poor mixing and heterogeneous staining distributions.
6. Ethanolic dye stock should not be added directly to the cell pellet. This will result in poor cell viabilities.
7. Rapid and homogeneous mixing is critical for uniform labeling. The following measures have been found to aid in achieving optimum results:
 - Mix equal volumes of cell suspension and working dye solution;
 - Avoid staining in very small (<100 µL) or very large (>5 mL) volumes.
 - Avoid the use of serological pipettes for the addition of cells to dye.
 - Dispense volumes as precisely as possible so that both cell and dye concentrations are accurately reproduced from sample to sample and study to study.
8. 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.
9. Do not centrifuge the cells in Diluent C before stopping the staining reaction.
10. Staining should be stopped by adding an equal volume of serum or other suitable protein source. Serum is preferred.
11. 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 10).
12. Do not use Diluent C for washing steps.
13. 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, 7, 8).
14. 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.
15. Stained cells may be fixed with 2% paraformaldehyde and intensities are expected to be stable for several weeks so long as the samples are protected from direct bright light.
16. 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 (PolyFreeze, Cat. #19636)
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 Crazy Glue).
7. Examine or photograph sections using standard filter setup.

Should any of our materials fail to perform to our specifications, we will be pleased to provide replacements or return the purchase price. We solicit your inquiries concerning all needs for life sciences work. The information given in this bulletin is to the best of our knowledge accurate, but no warranty is expressed or implied. It is the user's responsibility to determine the suitability for his own use of the products described herein, and since conditions of use are beyond our control, we disclaim all liability with respect to the use of any material supplied by us. Nothing contained herein shall be construed as a recommendation to use any product or to practice any process in violation of any law or any government regulation

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 Mayers (Cat. #24821) or Harris hematoxylin (Cat. #24245).
4. Mount slides using AS/AP permanent aqueous mounting medium (Aqua-Poly/Mount, Cat. #18606).

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 (12). For a protocol successfully used to detect PKH26, a visible-emitting membrane dye, in combination with an immunohistochemical counterstain, see Soukiasian et al (16).

Storage/Stability

- CellVue® 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 for general Cell Membrane Labeling).
- 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.

References

1. Horan, P. K., and Slezak, S. E., Nature, 340, 167-168 (1989)
2. Horan, P.K., et al., Methods Cell Biol., 33, 469-490 (1990)
3. Poon, R.Y., et al., in: In Living Color: Flow Cytometry and Cell Sorting Protocols, Diamond, R. A., and DeMaggio, S. (Eds.). p. 302-352 (Springer-Verlag, New York, 2000)
4. Al-Mehdi, A-B, Patel M, Haroon A, Reed D, Ohlsson-Wilhelm B, Muirhead K and Gray BD. Increased Depth of Cellular Imaging in the Intact Lung Using Far Red and Near Infrared Fluorescent Probes. Int. J. Biomed. Imag., Vol 2006, Article ID 37470, p1-7.
5. Bantly AD, Gray BD, Breslin E, Weinstein EG, Muirhead KA, Ohlsson-Wilhelm BM, Moore JS. CellVue® Claret, a new Far-Red Dye, Facilitates Polychromatic Assessment of Immune Cell Proliferation. Immunological Investigations, 36, No 5-6, 581-605 (2007)
6. Tario, JD, Gray BD, Wallace SS, Muirhead KA, Ohlsson-Wilhelm BM, Wallace PK. Novel Lipophilic Tracking Dyes for Monitoring Cell Proliferation. Immunological Investigations, 36, No 5-6, 861-885 (2007)
7. Barbier M, Gray BD, Muirhead KA, Ronot X and Boutonnat J. Cytometry Part B: Clinical Cytometry 59B, 46-53 (2004).
8. Al-Mehdi, A-B, Patel M, Haroon A, Reed D, Ohlsson-Wilhelm B, Muirhead K and Gray BD. Increased Depth of Cellular Imaging in the Intact Lung Using Far Red and Near Infrared Fluorescent Probes. Int. J. Biomed. Imag., Vol 2006, Article ID 37470, p1-7.
9. Gertner-Dardenne J, Poupot M, Gray B and Fournie JJ. Lipophilic Fluorochrome Trackers of Membrane Transfers between Immune Cells. Immunological Investigations. 36, Nos 5-6, 665-685 (2007)
10. Stewart, C. C., M. L. Woodring, et al. (2005). "Flow cytometer in the infrared: inexpensive modifications to a commercial instrument." Cytometry A 67(2): 104-11.
11. Dr. Edward Roy, University of Illinois, personal communication.
12. Wallace P.K., et al., Cancer Res., 53, 2358-2367 (1993)
13. Mitchell, E.A. Eur. J. Immunol., 28, 3066-3074 (1998)
14. Maus, U., et al., Am. J. Physiol. Lung Cell Mol. Physiol., 280, L58-68 (2001)
15. Albertine, K.H., Gee, M.H. J. Leukoc. Biol., 59, 631-638 (1996)
16. Soukiasian HJ et al. J. Heart Lung Transplant. 23: 873-880 (2004)

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

Should any of our materials fail to perform to our specifications, we will be pleased to provide replacements or return the purchase price. We solicit your inquiries concerning all needs for life sciences work. The information given in this bulletin is to the best of our knowledge accurate, but no warranty is expressed or implied. It is the user's responsibility to determine the suitability for his own use of the products described herein, and since conditions of use are beyond our control, we disclaim all liability with respect to the use of any material supplied by us. Nothing contained herein shall be construed as a recommendation to use any product or to practice any process in violation of any law or any government regulation

Ordering Information

Cat. #	Description	Size
24850-1	CellVue® Burgundy Kit For Membrane Labeling - Midi Kit	1 Kit
24843-1	CellVue® Burgundy Mini Kit For Membrane Labeling	1 Kit
24849-1	CellVue® Claret Kit For Membrane Labeling - Midi Kit	1 Kit
24844-1	CellVue® Claret Mini Kit For Membrane Labeling	1 Kit
24905-1	CellVue® Jade Kit For Membrane Labeling - Midi Kit	1 Kit
24904-1	CellVue® Jade Mini Kit For Membrane Labeling	1 Kit
24851-1	CellVue® Lavender Kit For Membrane Labeling - Midi Kit	1 Kit
24841-1	CellVue® Lavender Mini Kit For Membrane Labeling	1 Kit
24847-1	CellVue® Maroon Kit For Membrane Labeling - Midi Kit	1 Kit
24840-1	CellVue® Maroon Mini Kit For Membrane Labeling	1 Kit
24852-1	CellVue® NIR780 Kit For Membrane Labeling - Midi Kit	1 Kit
24845-1	CellVue® NIR780 Mini Kit For Membrane Labeling	1 Kit
24853-1	CellVue® NIR815 Kit For Membrane Labeling - Midi Kit	1 Kit
24846-1	CellVue® NIR815 Mini Kit For Membrane Labeling	1 Kit
24848-1	CellVue® Plum Kit For Membrane Labeling - Midi Kit	1 Kit
24842-1	CellVue® Plum Mini Kit For Membrane Labeling	1 Kit

Additional Products:

19636	PolyFreeze (Frozen Mounting Medium) 6x120ml	1 Case
18606	Aqua-Poly/Mount	20ml, 100ml, 5x20ml
24821	Mayers hematoxylin	500ml, 1 Liter
24245	Harris hematoxylin	500ml, 1000ml

Sold under sublicense from PTI Research, Inc. to MTTI.
CellVue® is a trademark of PTI Research, Inc.
U.S. Patent #5,665,328

To Order In The US:

In The U.S. Call: 1-800-523-2575 • (215) 343-6484
In The U.S. Fax: 1-800-343-3291 • (215) 343-0214

To Order In Germany:

In Germany Call: (49) 6221-765767
In Germany Fax: (49) 6221-764620

Visit our website to order online anytime at www.polysciences.com

Should any of our materials fail to perform to our specifications, we will be pleased to provide replacements or return the purchase price. We solicit your inquiries concerning all needs for life sciences work. The information given in this bulletin is to the best of our knowledge accurate, but no warranty is expressed or implied. It is the user's responsibility to determine the suitability for his own use of the products described herein, and since conditions of use are beyond our control, we disclaim all liability with respect to the use of any material supplied by us. Nothing contained herein shall be construed as a recommendation to use any product or to practice any process in violation of any law or any government regulation