cell signaling

PKH Linker Kits for Fluorescent Cell Labeling

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Application Notes

- Achieve stable, uniform, intense, and reproducible fluorescent labeling of live cells
- Stable labeling for up to 100 days
- Non-cytotoxic with no effect on biological or proliferative activity
- Intense and easy to use
- Versatile – use with any cell type
- Minimal leaking or transfer from cell to cell

Introduction

Many key research findings in cell function and pathogenesis have relied on labeling live cells. To study individual cell behavior, growth and differentiation, and cell-cell interactions, it is critical to be able to permanently "tag" a population of cells without affecting their morphology or function. Studies of cellular phenomena like adhesion, conjugate formation, cytotoxicity, phagocytosis, growth promotion, survival time, apoptosis, and hybridoma fusion all rely on dependable nontoxic, stable cell labeling.

Early methods for labeling cells employed fluorescent labels such as fluorescein, rhodamine, DiI-C_{18}-(3), or Hoechst dyes; or radiolabels like ^{125}I-idoxuridine, ^{111}In-indium oxine and ^{51}Cr-sodium chromate. These methods served well in their time, but each has limitations and drawbacks.[1] Some are not stable enough, so they leak and transfer to other cells in a mixed population, confounding results. Others alter cell function or are cytotoxic, and thus cause experimental artifacts related directly to their use. Finally, these methods often label weakly or non-uniformly, making detection difficult and design of reliable labeling protocols impossible.

PKH Fluorescent Cell Linker Kits avoids all the pitfalls of these traditional methods. Now there is a cell labeling method that is the single "best way" to consistently and reliably label viable cells (Table 1). Named for their discoverer, Paul Karl Horan, PKH dyes are patented fluorescent dyes and cell labeling technology[2] has been used successfully with animal, plant, and bacterial cells, and even non-cellular membrane-containing particles.[3] Labeled cells can be studied in culture and *in vivo*. Rapidly dividing cells like hybridomas, as well as non-proliferative cells like red blood cells, can be labeled and tracked.

PKH fluorescent dyes offer advantages

When you buy from Sigma, you get the PKH dyes, the specially formulated diluents, and the tested labeling protocols all in kit form. We've already determined the optimum combinations of reagents and conditions for intense, fast and reproducible viable-cell labeling in many model systems. Our kits make PKH labeling easy.

Versatile

The versatility of PKH cell-labeling technology is attributable to its innovative chemistry. Intensely fluorescent dye moieties are attached to long, lipophilic tails. During the short general membrane staining procedure – only 1-5 minutes – the lipophilic tails diffuse into the cell membrane, leaving the fluorogenic moiety exposed near the outer surface of the cell.

Stable

The stable partitioning of these molecules into the membrane permits long-term monitoring while leaving the important functional surface proteins unaltered. In fact, all cellular functions, from proliferation to cell-surface antigen recognition, are largely unaffected by PKH dye tagging. In short, labeled cells behave just like unlabeled ones; they're just easier to track.

Intense, reproducible membrane staining

PKH dye labeling protocols can usually be optimized to give over 75% viable labeled cells with mean fluorescence more than 1,000 times background (Figure 1). Such high signal-to-noise ratios are especially important when tagging proliferating cell lines because each daughter cell gets only half its parent's label. With PKH dyes, up to ten generations can be followed before the intensity of the label falls near background.

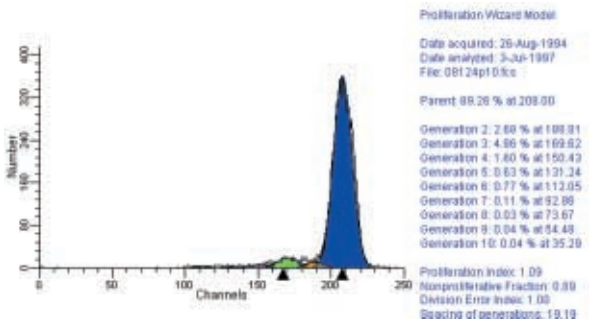

Figure 1. Deconvoluted histogram of PKH26-labeled human PBL on Day Zero showing non-proliferated control parent population.

Characteristics of PKH dyes

Three different PKH dyes are used for labeling cells – PKH2, PKH26, and PKH67. PKH2 and PKH67 are green fluorescent labels; both can be detected with microscopes and flow cytometers equipped with fluorescein filters. Since their emission spectra have little overlap with the red region, they are ideal for cytotoxicity studies[4,5] in conjunction with red fluorescent viability probes like propidium iodide (PI) and 7-aminoactinomycin D (7-AAD), as well as with other red labels like R-phycoerythrin and Texas Red. They are also excellent for plant studies because they avoid interference from chlorophyll auto-fluorescence in the red region.

The *in vivo* half-life of PKH2 is 5-8 days, making it excellent for short- to medium-term studies. PKH67 has longer aliphatic tails than PKH2, resulting in more stable labeling and less cell-to-cell transfer.[6] It is expected to have an *in vivo* half-life of 10-12 days. Previous users of PKH2 may find that PKH67 gives even better results for longer-term studies and for studies in which it is essential to minimize cell-to-cell transfer (Figure 2).

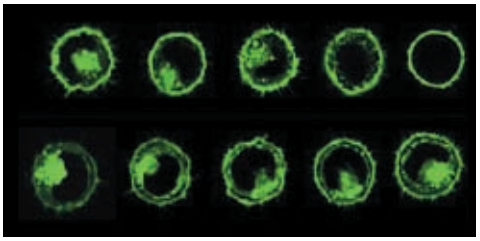

Figure 2. Internalization kinetics of PKH67 in L929 cells (Courtesy of Cecile Rousselle and Dr. Xavier Ronot, Universite Joseph Fourier, Grenoble, France).

PKH26 is a red fluorescent dye. It offers the longest *in vivo* half-life – greater than 100 days – making it ideal for *in vivo* cell tracking, cell proliferation studies, and other long-term assays.[7-9] Rhodamine or phycoerythrin (PE) filters are suitable for PKH26 detection. Standard fluorescein excitation wavelengths may be used, but fluorescence intensity will be somewhat reduced.

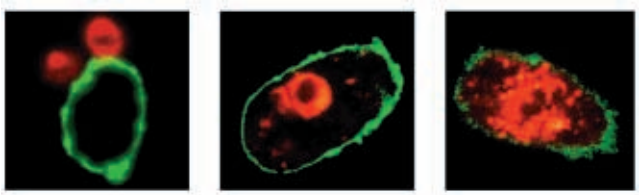

Figure 3. Confocal assay for phagocytosis of malignant lymphocytes by monocytes derived macrophages. *Macrophages (labeled with green with FITC). Malignant lymphocytes (stained red with PKH26) and bispecific antibodies were combined and incubated at 37 °C. Confocal sections were examined after 30, 60 and 360 minutes and illustrate the process of target binding, ingestion and destruction. Ely, P., Wallace, P.K., Givan, A., et al., "Bispecific-armed interferon gamma primed macrophage mediated phagocytosis of malignant non-Hodgkins lymphome," Blood, 87, 3813 (1996). Reprinted with permission.*

PKH26 is complementary to the green PKH dyes. It can be quantitated without interference from PKH2, PKH67, or fluorescein-tagged antibodies in studies of mixed cell populations (Figure 3). Sigma's Cell Census Plus™ System builds on the unique properties of PKH26: using flow cytometry, you can measure proliferation and quantitate different phenotypes simultaneously in a mixed cell population.[8,10]

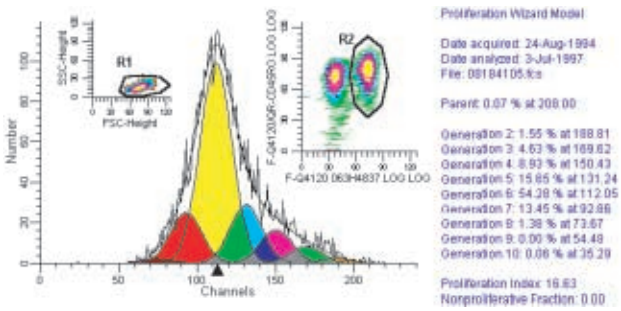

Figure 4. Deconvoluted histogram of $CD4^+/CD45RO^+$ subset in PKH26-labeled PBL activated with 2.5 μg/ml PHA for 6 days. *Inset shows gates set by light scatter and immunophenotype.*

Methods

For general cell labeling

The *in vitro* general cell labeling procedure can be used for monocytes, macrophages, lymphocytes, and other cells in suspension. Cells grown on the surface of tissue culture vessels may be stained *in situ* but heterogeneous staining may result. For homogeneous staining, adherent cells should first be suspended with a proteolytic treatment (*e.g.,* trypsin + EDTA). Figure 5 represents the protocol schematically.

A 2X cell suspension and a 2X dye solution, both in the PKH diluent supplied with the kit, are mixed and incubated briefly at room temperature. The labeling reaction is stopped by addition of protein (medium with serum or BSA). Labeled cells are washed 3-5 times to remove unbound dye. General cell labeling should be performed prior to monoclonal antibody staining to avoid capping the antibody with the dye.

For phagocytic cell labeling

Phagocytic cells can be selectively labeled in the prescence of non-phagocytic cells by using an alternate diluent. In this diluent, dyes do not dissolve, but instead form microaggregates; these microaggregates cannot become embedded in membrane, but can be ingested by phagocytosis. This method has been used to study lifetime and migrational patterns of macrophages and neutrophils *in vivo*.

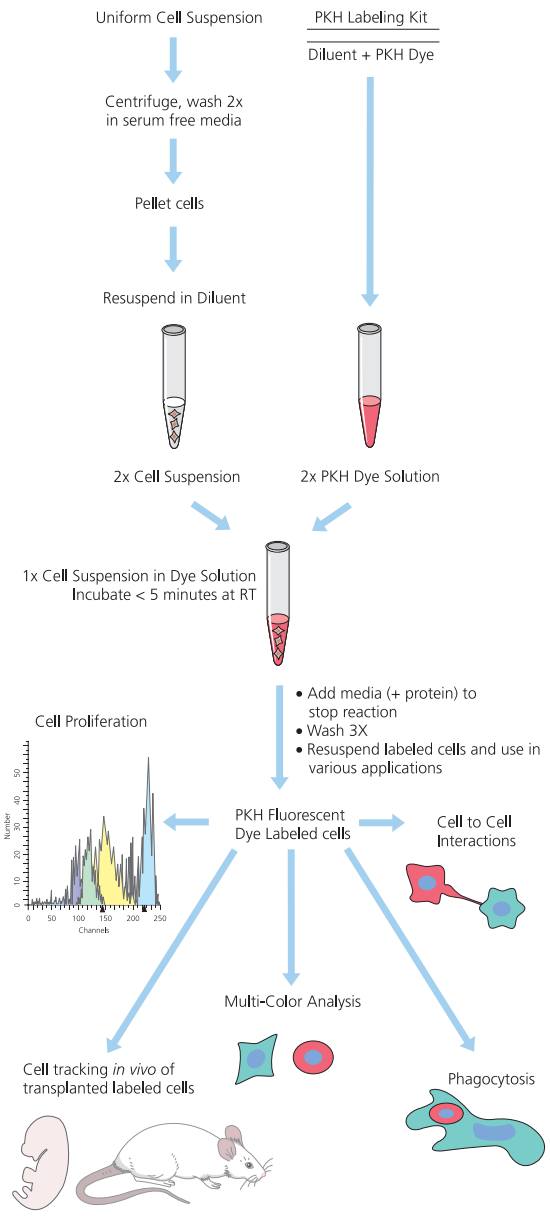

Figure 5. Standard protocol for PKH dye labeling with Sigma kits.

Summary

PKH Fluorescent Cell Linker Kits use patented fluorescent cell linker technology to incorporate reporter molecules into the cell membrane. Labeled cells retain both biological and proliferative activity, and are ideal for cell tracking and cell-cell interaction studies. Due to the non-specific labeling of the dyes, a wide variety of cell types have been labeled successfully. The linkers have been applied to both animal and plant cells. Table 2 lists some common cell types that have been successfully labeled and Table 3 lists many PKH dye applications.

References

1. Samlowski, W.E., et al., *J. Immunol. Methods,* **144,** 101 (1991).
2. The patented PKH dye technology, developed by Zynaxis Cell Science, is now owned by Phanos Technologies.
3. Horan, P.K., et al., *Methods in Cell Biology,* **33,** 469 (1990).
4. Slezak, S. and Horan, P., *J. Immunol. Meth.,* **177,** 205 (1989).
5. Hatam, L., et al., *Cytometry,* **16,** 59 (1994).
6. Unpublished data, Zynaxis, Inc.
7. Horan, P. and Slezak, S., *Nature,* **340,** 167 (1989).
8. Yamamura, Y., et al., *Cell. Molec. Biol.,* **41,** S121 (1995).
9. Wallace, P., et al., *Cancer Res.,* **53,** 2358 (1993).
10. Hendrikx, P., et al., *Exper. Hematol.,* **24,** 129 (1996).

Ordering Information

Product	Description	Unit
PKH26-GL	PKH26 Red Fluorescent Cell Linker Kit for General Cell Labeling	1 kit
MINI-26	PKH26 Red Fluorescent Cell Linker Mini Kit for General Cell Labeling	1 kit
PKH26-PCL	PKH2 Red Fluorescent Cell Linker Kit for Phagocytic Cell Labeling	1 kit
PKH67-GL	PKH67 Green Fluorescent Cell Linker Kit for General Cell Labeling	1 kit
MINI-67	PKH67 Green Fluorescent Cell Linker Mini Kit for General Cell Labeling	1 kit
PKH2-GL	PKH2 Green Fluorescent Cell Linker Kit for General Cell Labeling	1 kit
PKH2-PCL	PKH2 Green Fluorescent Cell Linker Kit for Phagocytic Cell Labeling	1 kit
CCPS-1	Cell Census Plus™ System, a Cell Proliferation Assay for Flow Cytometry	1 kit

Table 1.

Desired Characteristic	Lipid Intercalators		Covalent Protein Tags		Genetic Markers	Radio-labels
	PKH Dyes	DiI, DiO	CFSE	Biotin-SE	GFP, β-Gal	In-111, Cr-51
Fast, simple labeling	+++	varies	+++	++	–	+
Intense labeling	++++	varies	++++	++++	varies	+
Uniform and reproducible	+++	varies	+++	+++	+	+
Applicable to most cell types	++++	varies	+++	+++	varies	varies
Not immunogenic	++++	++++	+	+	++	++++
No effect on cell receptors or functions	++++	++	varies	varies	+	–
Stable over many generations	++	++	++	+	+++	–
Immediate proliferation monitoring possible	++++	++++	24-48 hr delay	24-48 hr delay	–	varies
Easy to monitor distribution *in vivo*	+	+	+	+	+/–	+++

Abbreviations: DiI = 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate; DiO = 3,3′-dioctadecyloxacarbocyanine perchlorate; CFSE = carboxyfluorescein succinimidyl ester; Biotin-SE = biotin-succinimidyl ester; GFP = green fluorescent protein; β-Gal = beta-galactosidase

Table 2. Cell types successfully labeled with PKH dyes.

Hematopoietic cells: $CD34^+$ stem cells, CFU-s, bone marrow transplant "facilitating cells", long term culture initiating cells, primitive progenitor cells, stromal cells Other blood cells: ***Erythrocytes, neutrophils, platelets***	**Bacteria, viruses, and parasites:** *Bacillus subtilis, Haemophilus somnus, Leishmania donovani, Listeria monocytogenes, Plasmodium gallinaceum, Salmonella typhimurium, Schistosoma mansonii,* HIV, *Trichinella spiralis, Vibrio cholerae*
Immune cells: Lymphocytes, monocytes, macrophages, thymocytes, splenocytes, cord blood T cells, cytotoxic T cells, autoimmune T cells, NK cells, Langerhans and dendritic cells	**Non-mammalian cells/organisms:** *Dictyostelium discoideum* Newt lens-regenerating cells *Rana catesbeiana* (bull frog) red blood cells Zebrafish embryos
Other primary cells: Chick germ cells, embryonic cells, fibroblasts, epithelial cells, endothelial cells, leukemic blasts, mast cells, mesenchymal cells, myoblasts, neurons and neuronal precursors, smooth muscle cells	**Plant Cells** Protoplasts Phytoplankton
Cultured cells and cell lines: Hybridomas, T cell lines and clones, tumor cell lines (K562, HL60)	**Miscellaneous particles:** Erythrocyte ghosts Fluorocarbon emulsions Liposomes

Table 3. Monitoring cell functions using PKH dyes.

In vitro:	*In vivo:*
Apoptosis (in conjunction with Annexin V-FITC)	Migration
Differentiation	Homing
Drug sensitivity/resistance	Engraftment
Cell-cell communication	Immune reconstitution
Antigen-driven proliferation	Proliferation
Conjugate formation, adhesion, and/or fusion	Growth control
Cellular cytotoxicity	Differentiation
Bystander killing	Embryogenesis
Antitumor immunity	Adhesion
Effect of vaccination on frequency of antigen specific precursors	Blood flow
Phagocytosis	Antitumor immunity
	Antigen presentation