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Product Information

PKH2 Green Fluorescent Cell Linker Kit for Phagocytic Cell Labeling

Catalog Number **PKH2PCL**

TECHNICAL BULLETIN

Product Description

The PKH2-PCL fluorescent cell linker kit selectively labels cells with phagocytic capabilities such as macrophages or neutrophils using patented Zyn-linker technology.[1-3] Labeling occurs through formation of dye aggregates or particulates induced by Diluent B. As with PKH1[4-6] and PKH26,[7,8] dye aggregate formation significantly inhibits the uptake of dye by nonphagocytic cells, such as lymphocytes[1,3-5] or circulating monocytes[7], but facilitates dye uptake by phagocytic cells such as peritoneal or alveolar macrophages or circulating neutrophils. Labeled cells appear patchy or spotted because the dye is localized in phagocytic compartments of the cells.[4] The dye appears to be resistant to metabolic attack and has been found to remain with the cells for more than 28 days *in vivo*.[1]

Labeling of phagocytic cells by this methodology may be carried out either *in vitro* or *in vivo*. Intraperitoneal[1-6] or intravenous[7,8] injections of the PKH2 labeling solution will successfully label phagocytic cells *in vivo*, while cells of interest which have been isolated may be stained using *in vitro* labeling methods,[1,8]

Reagents

- PKH2 dye stock (1 vial containing >0.5 ml, 1×10^{-3} M in ethanol)
- Diluent B (6 vials, each containing >10 ml)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage

All reagents may be stored at room temperature or refrigerated. Dye stock should be kept tightly capped when not in immediate use to prevent evaporation.

Materials and Methods

Materials Required for *i.p*. Cell Labeling (Steps 1-7 of Procedure section):

1. Mice or other animal species whose phagocytic cells are to be labeled.
2. PKH2-PCL Fluorescent Cell Linker Kit
3. Absolute ethanol.
4. 20 cc sterile syringe with needle.
5. Sterile 1 cc syringes with 27 gauge 1-inch needles (1 per animal, plus 1 extra).
6. Bunsen burner or alcohol burner.
7. Sterile gauze squares.
8. 70% Ethanol in wash bottle.

Materials Required for Harvesting Labeled Peritoneal Macrophages (Steps 8-14 of Procedure section):

1. 70% Ethanol in wash bottle
2. Forceps, surgical scissors and peg board.
3. 5 cc syringe with 20 or 22 gauge needle
4. Dulbecco's phosphate buffered saline (PBS), (Ca^{+2} and Mg^{+2} free).
5. 15 cc polypropylene centrifuge tubes
6. Ice bucket with ice
7. Additional materials for immunofluorescent staining as determined by the user.
8. Instrument for analysis of fluorescence (fluorometer, fluorescence microscope, flow cytometer, or fluorescence image analysis instrumentation).

Procedure for *in vivo* Labeling of Resident Peritoneal Macrophages

This procedure has been optimized for labeling the resident peritoneal Mϕ of Balb/c mice, approximately 20 gm body weight. The peritoneum of naive Balb/c mice (housed under virus free conditions) contains 20-30% Mϕ.[4] Labeling conditions, including dye concentration and volume to be injected intra-peritoneally (*i.p.*), should be optimized for other mouse strains which are larger or have more peritoneal Mϕ.

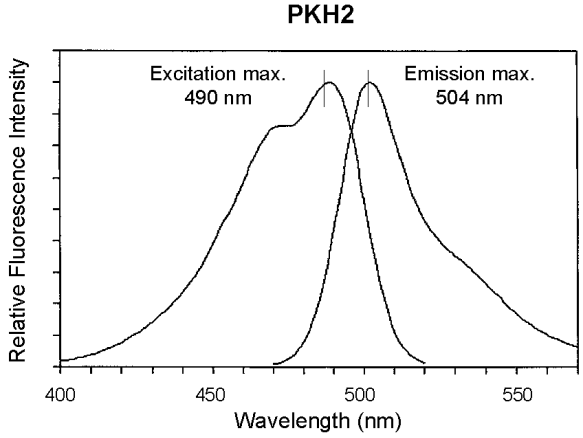
1. Remove 0.05 ml PKH2 (1 x 10^{-3} M) from kit and mix thoroughly with 0.90 ml absolute ethanol. This will provide 50 µM working stock dye solution. Dye stock should be kept tightly capped when not in immediate use to prevent evaporation.
2. Remove the metal tab of one diluent bottle. Because the diluent bottles have been overfilled to assure at least a 10 ml volume, withdraw the contents of the bottle using a 20 cc syringe with needle, expel the overfill volume and reinject 10 ml back into the vial.
3. Remove 0.1 - 0.2 ml of the working stock (50 µM) with a 1 cc syringe. Inject 0.05 ml of the dye into the diluent bottle. If the needle will not penetrate the rubber stopper, the stopper may be removed. However, it is essential that the contents of the diluent vial remain sterile as any contamination will induce inflammation into the peritoneal cavity.
4. Replace the stopper, if necessary, and shake the bottle vigorously to mix the dye and diluent. Allow the solution to stand for at least 15 minutes.
5. Withdraw 0.5 ml of the diluted dye into a fresh sterile syringe for each animal to be injected. If possible, keep the rubber stopper on the diluent vial and flame the stopper before inserting each needle. Flame the needle again after withdrawing the dye, then flame the needle cap and place it over the needle.
6. Repeat step 5 for each syringe to be filled. Use a fresh syringe and needle for each animal. Shake the dye/diluent mixture before removing each sample for injection.
7. Inject the contents of each syringe intraperitoneally (*i.p.*). Before each injection, swab the abdominal fur with a gauze pad soaked in alcohol. This procedure will minimize the incidence of inflammation from the *i.p*. injection.
8. Labeled peritoneal Mϕ may be harvested by peritoneal lavage at any time from 2 hours to 28 days after the i.p. injection of PKH2 dye. To harvest the peritoneal cells, sacrifice the animal (e.g. by cervical dislocation), and wet the abdominal fur with 70% ethanol.
9. Make a small incision in the inguinal area cutting through the fur, but **not** through the dermis. Grasp the abdominal fur and retract the fur towards the shoulders. This procedure will expose the intact abdominal cavity.
10. Fill a 5 cc syringe with ice-cold PBS (larger volumes -- up to 10 ml -- may be preferred for larger mice), forcefully inject the PBS into the abdominal cavity, and withdraw the syringe. The injection site should be the inguinal fat pads. The abdominal fat will seal the injection site when the syringe is withdrawn.
11. Massage the abdominal cavity to loosen peritoneal cells adhered to the abdominal wall and viscera.
12. Insert the needle into the abdominal cavity, about midline, and gently withdraw the lavage fluid. With some experience, the user will be able to collect 90% of the injected fluid.
13. Place the contents of the syringe into a 15 cc polypropylene tube. The tube should be kept on ice to minimize adherence and/or clumping of the Mϕ.
14. Centrifuge the peritoneal cells for 5 minutes at 350 x *g*, at 4 °C. The cells can then be re-suspended and washed in saline or immuno-fluorescence wash buffer (for immunofluorescent staining) or culture medium (for functional assays).

The procedure described is designed to selectively label resident peritoneal Mϕ of mice. The resident Mϕ (in the peritoneum at the time of injection) can be distinguished from subsequently recruited Mϕ, which are not labeled by the green fluorescent dye, PKH2. This procedure can be adapted to label other phagocytic cells, *e.g*. neutrophils or resident alveolar Mϕ, either *in vivo* or *in vitro*. The reagents in the PKH2-PCL Cell Linker kit have been formulated to favor selective labeling of phagocytic cells over non-phagocytic cells (*e.g*. lymphocytes) present in the population. Methods have also been described for monitoring phagocytic cell ingestion of PKH2-, PKH67- or PKH26-labeled targets such as bacteria,[9,10] liposomes,[11] red blood cells,[12,13] tumor cells,[14,15] and/or apoptotic vesicles.[16]

Critical Aspects of Phagocytic Cell Labeling

1. This procedure is designed to selectively label resident Mϕ. If monocytes/Mϕ are recruited to the peritoneum as the result of inflammation induced by the *i.p*. injection, they will probably not be labeled by the dye. The peritoneal cavity is very sensitive to inflammation and the procedure described above utilizes several precautions to minimize this inflammation including keeping the injection vial and syringes absolutely sterile, using a fresh disposable syringe and 27 gauge needle for each animal, and wiping the abdominal fur with alcohol before each injection.
2. In spite of the precautions described above, some injection-induced inflammation has been routinely observed in as many as 50% of the mice. This percentage will decline with practice. Injection induced inflammation is detected, following immunofluorescent labeling and flow cytometric analysis, as two populations of Mϕ (dye labeled and unlabeled), or one broad population of Mϕ with variable stain intensity, or very few to no labeled Mϕ in extreme inflammation.[2] At 24 hours after injection, animals with inflammation can be identified by elevated peritoneal cell number and elevated neutrophil counts, compared to untreated animals.
3. Extra animals may be injected for each study and after immunofluorescence pattern analysis and differential cell counts of each animal, data from the animals with inflammation is not included.
4. In the absence of inflammation, resident peritoneal Mϕ can be distinguished from recruited Mϕ, by their green fluorescence intensity, for up to 28 days *in vivo*.[3]
5. Diluent B has been tested for lipopolysaccharide (LPS) content and has been found to contain less than 0.0625 EU/ml.
6. The staining conditions should be optimized for each species (and strain) of animal tested. Contact Sigma Technical Service for further information.
7. The PCL staining kit can also be used to label phagocytic cells *in vitro*. The concentration of PKH2 for *in vitro* labeling should be much higher than that used for *in vivo* labeling.[1] The procedure described for general cell labeling can be adapted for labeling phagocytic cells *in vitro*, by using the PCL diluent in place of the GL diluent. Contact Sigma Technical Service to obtain a copy of the procedure for general cell labeling.

PKH2 Excitation and Emission Spectra



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