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Product Information

Troubleshooting Guide for PKH and CellVue® Dyes

Troubleshooting Guide for PKH26, PKH67, and CellVue® Claret Fluorescent Cell Linker Dyes		
	Problem	**Solution**
Cell Clumping (Refs. 1-4)	Dye concentration too high.	Reduce dye concentration to avoid over-labeling.
	Cells exposed to dye too long.	Stop staining after 1-5 minutes by adding serum or other protein.
	Serum not added to stop dye uptake.	Use serum, other protein or medium with at least 10% serum as stop reagent to avoid over-labeling.
	Too many platelets present in sample.	Centrifuge sample at low speed to remove platelets before staining.
	Adherent cells incompletely dispersed.	Use enzymatic or mechanical treatment (trypsin or aspiration through a needle) to prepare a single cell suspension for staining.
	Poor cell viability in sample used for staining.	Incubate with 0.002 % DNase for 30 minutes at 37 °C before staining.
Poor Staining Intensity (Ref. 1-6)	Filters incorrect for observing dye.	Verify cell labeling and check filter set-up. If pellet is visibly pink (PKH26), yellow (PKH67), or blue (CellVue Claret), cells are stained.
	2× dye stock prepared too long before adding to cells - dye aggregation reduces staining efficiency.	Prepare 2× working dye stock in Diluent C immediately prior to initiation of labeling by addition of 2× cells in Diluent C.
	Serum present during labeling binds dye and reduces staining efficiency.	Wash cells in serum-free buffer 1-2 times prior to resuspension in Diluent C for labeling.
	Salt content of labeling solution too high - dye aggregation reduces staining efficiency.	Aspirate as much supernatant as possible from washed cell pellet before resuspending in Diluent C. DO NOT aspirate cells.
	Staining intensity varies with cell type based on cell size, membrane surface area, and/or membrane composition.	Adjust dye concentration for each cell type as needed to reach intensity required for specific experimental application. Carry out subset analysis on the cell type(s) of interest..
	Not enough dye for amount of cells used.	Increase dye concentration and/or decrease cell concentration.
	Cell number too high for amount of dye used.	Reduce cell concentration and/or increase dye concentration.
Patchy or Punctate Staining (Ref. 1-3,6,7)	Salt content of labeling solution too high – dye aggregates form, reducing staining efficiency.	Aspirate as much supernatant as possible from buffer-washed cell pellet before resuspending in Diluent C. DO NOT aspirate cells.
	Redistribution of dye due to membrane trafficking after initial labeling of plasma membrane.	Dependent on cell type and activation state. Not expected to alter total fluorescence intensity per cell.
Heterogeneous Staining (Ref. 1-4)	Different cell types in sample - staining intensity varies from cell type to cell type.	Isolate cell type(s) of interest before labeling OR carry out subset analysis on the cell type(s) of interest.
	Heterogeneous exposure to dye during staining.	Optimize method of admixing to ensure rapid and homogeneous dispersion of 2× cells (in Diluent C) into 2× dye (in Diluent C). Use enzymatic or mechanical treatment (trypsin or aspiration through a needle) to prepare a single cell suspension for staining.
	Not enough dye for number of cells used.	Increase dye concentration and/or decrease cell concentration.
	Cell concentration too high for amount of dye used.	Reduce cell concentration and/or increase dye concentration.
	Serum present during labeling interfering with dye incorporation.	Wash cells in serum-free buffer 1-2 times prior to resuspension in Diluent C for labeling.
	Dye loss on tube walls reduces staining efficiency.	Use only polypropylene tubes, other plastics adsorb dye.
	Cells clumped before exposure to dye.	Carefully aspirate sample through a needle or triturate cells in medium or buffer prior to resuspenson in Diluent C for staining.
Cells Dead (Ref. 1-5)	Dye concentration too high.	Reduce dye concentration to avoid over-labeling.
	Ethanol concentration too high	Keep final ethanol concentration in staining step to ≤2%
	Toxicity from serum used to stop staining.	Heat-inactivate serum at 56 °C for 60 minutes to inactivate.
	Sensitivity of specific cell type to Diluent C.	Check viability of a Diluent C only (no dye) control.

Troubleshooting Guide for PKH26, PKH67, and CellVue Claret Fluorescent Cell Linker Dyes (continued)		
	Problem	**Solution**
Viability/ Function (Ref. 1-5)	Poor viability or altered cell function.	Keep final ethanol concentration in staining step to ≤2%. Excessive dye uptake can alter cell viability or function - reduce dye concentration and/or increase cell concentration.
	Poor cell recovery after staining.	Do not decant supernatants - use a small pipette tip (P20) to aspirate. Excessive dye uptake can alter cell recovery - reduce dye concentration and/or increase cell concentration.
	Difficulty resuspending pelleted cells because of clumping in bottom of test tube.	Excessive dye uptake can alter cell viability - reduce dye concentration and/or increase cell concentration. Incubate cells with 0.002 % DNase for 30 minutes at 37 °C after staining is stopped but before centrifuging.
	Cells appear shriveled or swollen when viewed in microscope.	Non-mammalian cells may require special diluent formulations - call Sigma Technical Service.
Dye Loss/ Transfer (Refs. 1-5, 8)	Dye transfer to unstained cells in co-culture.	Use neat serum or buffer with 5% albumin to stop staining. Do not use protein free buffer or medium with <10% serum to stop staining -- addition of physiologic salts without sufficient protein to adsorb all unbound dye can cause formation of dye aggregates that pellet with stained cells and carry over into co-culture. Transfer sample to fresh tube during first post-staining wash. Wash twice with buffer or medium containing ≥10% serum. Culture stained cells in medium containing 10% serum for at least 30 minutes before addition to co-culture.
	Effector cells become positive for membrane dye used to label target cells.	Use flow cytometry or microscopy to check for: 1) Phagocytosis of target cells.by effectors (e.g., monocytes) 2) Trogocytosis of target cell membrane by effector cells. 3) Target cell apoptosis and uptake of labeled apoptotic vesicles by effector cells
	Polar organic solvents or detergents that extract membrane lipids can also extract dye	Use aqueous-based fixatives (methanol-free formalin) and solvents where possible.
Fixation of tissue sections (Refs. 3, 8)	Frozen sections show no fluorescence.	Use dry ice to freeze tissue for sectioning. Avoid use of polar organic solvents or detergents that extract membrane lipids – they may also extract dye.
	Low fluorescence intensity.	Be sure that sections are dry before adding glue and allow glue to dry before viewing - see Product Bulletin. Select cell linker dye that emits in a wavelength range where tissue autofluorescence is lower (typically in the red or far red).
	Histologic counterstain absorbs fluorescence.	Use serial sections OR use a single section and perform microscopy before counterstaining for morphology - see Product Bulletin.

Methods and Applications: References

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