CellTrace™ Cell Proliferation Kits

Catalog nos. C34554, C34557, C34564, C34567, C34568, C34570, C34571, C34572, C34573, C34574

Pub. No. MAN0002595 **Rev.** E.00

Product information

The CellTrace™ Cell Proliferation Kits provide versatile and well-retained cell tracing reagents in a convenient and easy-to-use form. Each kit contains a CellTrace™ reagent in single-use vials to permit small scale experiments without preparing excess quantities of stock solution. The CellTrace™ reagents readily diffuse into cells and bind covalently to intracellular amines, resulting in stable, well-retained fluorescent staining that can be fixed with aldehyde fixatives. Excess unconjugated reagent passively diffuses to the extracellular medium, where it can be quenched with complete media and washed away.

Table 1. Contents and storage

Material	Amount	Storage	Stability
CellTrace™ Blue (Component A)	9 vials (Cat. No. C34568)	• ≤–20°C • Desiccate • Protect from light	When stored as directed, the product is stable for at least 1 year.
	1 vial (Cat. No. C34574)		
CellTrace™ Violet (Component A)	9 vials (Cat. No. C34557)		
	1 vial (Cat. No. C34571)		
CellTrace™ CFSE (Component A)	10 vials (Cat. No. C34554)		
	1 vial (Cat. No. C34570)		
CellTrace™ Yellow (Component A)	9 vials (Cat. No. C34567)		
	1 vial (Cat. No. C34573)		
CellTrace™ Far Red (Component A)	9 vials (Cat. No. C34564)		
	1 vial (Cat. No. C34572)		
DMSO (Component B)	500 µL (Cat. Nos. C34554, C34557, C34564, C34567, C34568)	≤–20°C	
	100 µL (Cat. Nos. C34570, C34571, C34572, C34574)		
Number of reactions: Sufficient material is supplied for 20 reactions per vial of CellTrace™ Reagent, based on the protocol below.			
Approximate fluorescence excitation/emission maxima: See Table 2, page 2.			

Table 2. Approximate excitation and emission peaks of CellTrace™ reagents after hydrolysis.

CellTrace™ reagent	Cat. No.	Ex/Em maxima (after hydrolysis)	Recommended excitation source for flow cytometry
CellTrace™ Blue	C34568, C34574	355/410 nm	355-nm or 375-nm
CellTrace™ Violet	C34557, C34571	405/450 nm	405-nm
CellTrace™ CFSE	C34554, C34570	492/517 nm	488-nm
CellTrace™ Yellow	C34567, C34573	555/580 nm	532-nm or 561-nm
CellTrace™ Far Red	C34564, C34572	630/661 nm	633/635-nm

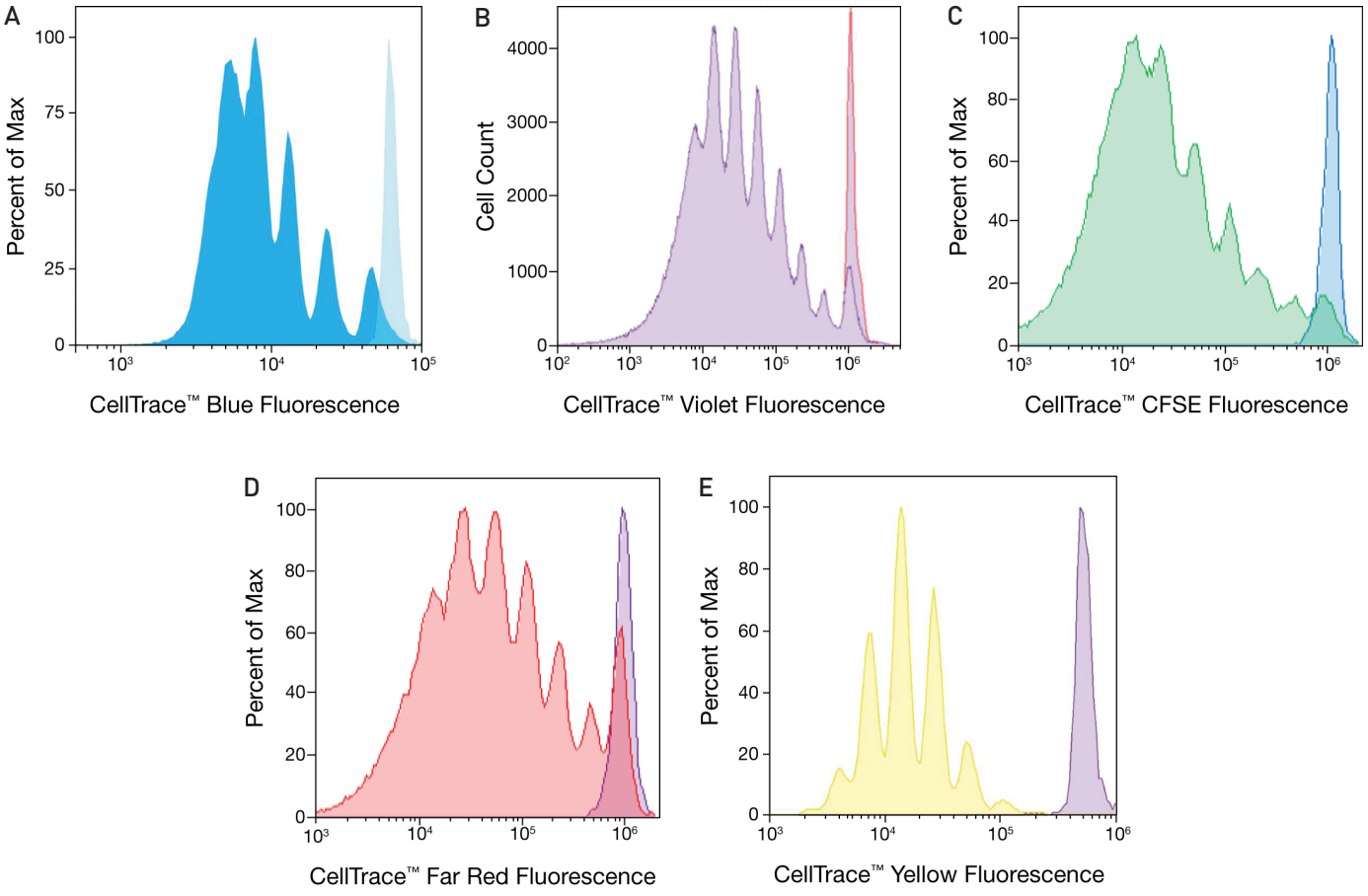
Figure 1. Generational tracing using CellTrace™ Reagents.

(A) Cell proliferation was followed for 5 days using the CellTrace™ Blue reagent. Human peripheral blood mononuclear cells were harvested and stained with 10 µM CellTrace™ Blue reagent prior to stimulation with Dynabeads™ Human T-activator CD3/CD28 (Cat. No. 111-41D). The discrete peaks in this histogram represent successive generations of live, CD4 positive cells. Acquisition was completed using a BD™ LSR II Flow Cytometer with 355-nm excitation and a 450/50-nm bandpass emission filter. An overlay of the unstimulated parent generation is indicated as the brightest peak (light blue) on the far right side of the histogram.

(B) Cell proliferation was followed for 8 generations using the CellTrace™ Violet reagent. Human peripheral blood mononuclear cells were harvested and stained with CellTrace™ Violet reagent prior to stimulation with mouse anti-human CD3 (Cat. No. MHCD0300) and Interleukin-2 (Cat. No. PHC0027) for 7 days. The discrete peaks in this histogram represent successive generations of live, CD4 positive cells. Analysis was completed using an Attune™ NxT Acoustic Focusing Cytometer with 405-nm excitation and a 450/40-nm bandpass emission filter. The unstimulated parent generation is indicated in red.

(C) and **(D)** Cell Proliferation was followed for 7 generations using the CellTrace™ CFSE and CellTrace™ Far Red reagents, respectively. Human T lymphocytes were harvested and stained with CellTrace™ CFSE or CellTrace™ Far Red reagent prior to stimulation with anti-human CD3 (Cat. No. MHCD0300) for 5 days. The discrete peaks in these histograms represent successive generations of live, SYTOX™ Green (Cat. No. S34860) negative cells. The unstimulated parent generation is indicated in blue (CellTrace™ CFSE), or purple (CellTrace™ Far Red). Analysis was completed using an Attune™ NxT Acoustic Focusing Cytometer with 488-nm excitation and a 530/30-nm bandpass emission filter for CellTrace™ CFSE. Analysis was completed for CellTrace™ Far Red using an Attune™ NxT Acoustic Focusing Cytometer with 638-nm excitation and a 660/20-nm bandpass emission filter.

(E) Cell proliferation was followed for 6 days using the CellTrace™ Yellow reagent. Human peripheral blood mononuclear cells were harvested and stained with 10 µM CellTrace™ Yellow reagent prior to stimulation with Dynabeads™ Human T-Activator CD3/CD28 (Cat. No. 111-41D). The discrete peaks in this histogram represent successive generations of live, CD4 positive cells. Acquisition was completed using an Attune™ NxT Acoustic Focusing Cytometer with 561-nm excitation and a 585/16-nm bandpass emission filter. An overlay of the unstimulated parent generation is indicated as the brightest peak on the far right side of the histogram.



Before starting

Materials required but not provided

- Cells of interest as a single-cell suspension
- Phosphate-buffered saline (PBS) or similar protein-free buffer
- Culture media containing protein such as FBS or BSA
- Flow cytometer

Caution

No data are available addressing the mutagenicity or toxicity of CellTrace™ reagents (Component A). Handle the DMSO dye solution with caution because DMSO is known to facilitate the entry of organic molecules into tissues. Always wear suitable protective clothing, gloves, and eye/face protection when handling this reagent. Dispose of the reagents in compliance with all pertaining local regulations.

Storage and handling

Upon receipt, store the kit components desiccated at ≤–20°C until required for use. When stored properly DMSO and dry CellTrace™ reagents are stable for at least one year. Allow the product to warm to room temperature before opening vials. Use stock solutions of CellTrace™ reagents only on the day of preparation. Avoid repeated freezing and thawing of kit contents.

Experimental protocols

General guidelines

- The following methods have been optimized for monitoring cell proliferation of human T and B lymphocytes.
- In other cell types and applications, we recommend titration of the CellTrace™ reagents to determine the optimal staining concentration. CellTrace™ stock solutions may be diluted in DMSO for this purpose.
- Recommended concentration for CellTrace™ staining is 1–10 µM. A dye concentration of 5–10 µM is recommended for tracking five or more generations, while 1–2 µM may be sufficient for tracking less than five generations.

  Note: Using the recommended concentration of 5–10 µM, CellTrace™ Blue reagent tracks up to 5 generations only.
- Start with a single cell suspension in PBS for uniform cell labeling.
- Uniform cell labeling can also be improved by gently agitating cells during staining.
- A low flow rate should be used for analysis on hydrodynamic focusing cytometers to ensure separation of distinct generational peaks. All collection rates will provide equivalent results when analyzing on an Attune™ NxT Acoustic Focusing Cytometer.
- To ensure appropriate instrument setup, include a sample of unstimulated control cells in proliferation experiments using CellTrace™ reagents.

Table 3. Preparation of CellTrace™ reagent stock solutions

Reagent	DMSO	Stock concentration	Working concentration
CellTrace™ Blue dye	40 µL	5 mM	5 µM
CellTrace™ Violet dye	20 µL	5 mM	5 µM
CellTrace™ CFSE dye	18 µL	5 mM	5 µM
CellTrace™ Yellow dye	40 µL*	5 mM*	5 µM
CellTrace™ Far Red dye	20 µL	1 mM	1 µM
*Prepare the stock solution at the specified concentration; using a higher concentration could result in a precipitate.			

Label cells in suspension

The following protocol has been optimized for cell concentrations of up to 10^6 cells/mL. Dye concentration may need to be increased for samples with $>10^6$ cells/mL.

1.1 Prepare CellTrace™ stock solution immediately prior to use by adding the appropriate volume of DMSO (Component B) to one vial of CellTrace™ reagent (Component A) and mixing well (see Table 2, page 3).

1.2 Add 1 µL of CellTrace™ stock solution in DMSO (prepared in Step 1.1) to each mL of cell suspension in PBS for a final working solution (see Table 3, page 3, for concentration).

1.3 Incubate the cells for 20 minutes at room temperature or 37°C, protected from light.

1.4 Add five times the original staining volume of culture medium (containing at least 1% protein) to the cells and incubate for 5 minutes. This step removes any free dye remaining in the solution.

1.5 Pellet the cells by centrifugation and resuspend them in fresh pre-warmed complete culture medium.

1.6 Incubate the cells for at least 10 minutes before analysis to allow the CellTrace™ reagent to undergo acetate hydrolysis.

1.7 Proceed with cell stimulation, incubation, or analysis.

Alternate method to label cells in suspension

The following protocol has been optimized for cell concentrations up to 10^6 cells/mL. Dye concentration may need to be increased for samples with $>10^6$ cells/mL.

2.1 Prepare CellTrace™ stock solution immediately prior to use by adding the appropriate volume of DMSO (Component B) to one vial of CellTrace™ reagent (Component A) and mixing well (see Table 2, page 3).

2.2 Pellet the cells by centrifugation and remove the supernatant.

2.3 Dilute the CellTrace™ DMSO stock solution in pre-warmed (37°C) phosphate-buffered saline (PBS) or other protein-free buffer to the desired working concentration (1–10 µM).

2.4 Gently resuspend the cells in the PBS dye solution (prepared in Step 2.1).

2.5 Incubate the cells for 20 minutes at room temperature or 37°C, protected from light.

2.6 Add five times the original staining volume of culture medium (containing at least 1% protein) to the cells and incubate for 5 minutes. This step removes any free dye remaining in the solution.

2.7 Pellet the cells by centrifugation and resuspend them in fresh, pre-warmed complete culture medium.

2.8 Incubate the cells for at least 10 minutes before analysis to allow the CellTrace™ reagent to undergo acetate hydrolysis.

2.9 Proceed with cell stimulation, incubation, or analysis.

Alternate method to label adherent cells

3.1 Prepare CellTrace™ stock solution immediately prior to use by adding the appropriate volume of DMSO (Component B) to one vial of CellTrace™ reagent (Component A) and mixing well (see Table 2, page 3).

3.2 Grow the cells to the desired density on coverslips or flasks filled with the appropriate culture medium.

3.3 Dilute the CellTrace™ DMSO stock solution in pre-warmed (37°C) phosphate-buffered saline (PBS) or other protein-free buffer to the desired working concentration (1–10 µM). This is the loading solution.

3.4 Remove the culture medium from the cells and replace it with the loading solution (prepared in Step 3.3).

3.5 Incubate the cells for 20 minutes at 37°C.

3.6 Remove the loading solution, wash the cells twice with culture medium containing at least 1% protein, and replace with fresh, pre-warmed complete culture medium.

3.7 Incubate the cells for at least 10 minutes before analysis to allow the CellTrace™ reagent to undergo acetate hydrolysis.

3.8 Proceed with cell stimulation, incubation, or analysis.

Optional: Fix and permeabilize cells

4.1 Label the cells with CellTrace™ reagent according to one of the protocols listed above.

4.2 Before fixation, wash and resuspend the cells in PBS or other protein-free buffer.

4.3 Fix the cells for 15–20 minutes at room temperature using an aldehyde-based fixative such as paraformaldehyde, protected from light.

4.4 Wash the cells with PBS.

4.5 If desired, permeabilize the cells using any appropriate protocol. CellTrace™ reagents covalently bind to cells and will not wash out after permeabilization.

4.6 Following permeabilization, wash the cells with PBS.

4.7 Resuspend the cells in PBS prior to analysis.

How to combine CellTrace™ reagents with other fluorescent markers

5.1 Label the cells with CellTrace™ reagent according to one of the protocols listed above.

5.2 Resuspend the cells in a buffer appropriate for the subsequent staining applications.

5.3 Apply stains for immunophenotyping, DNA content, apoptosis, or other markers as recommended for each stain.

References

1. J Cell Biol 101, 610 (1985); **2.** J Cell Biol 103, 2649 (1986); **3.** J Immunol Methods 171, 131 (1994); **4.** J Exp Med 184, 277 (1996); **5.** J Immunol Methods 133, 87 (1990); **6.** Transplant Proc 24, 2820 (1992); **7.** Current Protocols in Cytometry, J. P. Robinson, Ed., (1998) pp 9.11.1-9.11.9.; **8.** Cytometry Part A, 79A: 95–101 (2011); **9.** Current Protocols in Immunology, R. Coico, Ed., (2008) pp 7.10.1-7.10.24.

Ordering information

Cat. No.	Product name	Unit size
C34568	CellTrace™ Blue Cell Proliferation Kit *for flow cytometry* *180 tests*	1 kit
C34574	CellTrace™ Blue Cell Proliferation Kit *for flow cytometry* *20 tests*	1 kit
C34557	CellTrace™ Violet Cell Proliferation Kit *for flow cytometry* *180 tests*	1 kit
C34571	CellTrace™ Violet Cell Proliferation Kit *for flow cytometry* *20 tests*	1 kit
C34554	CellTrace™ CFSE Cell Proliferation Kit *for flow cytometry* *180 tests*	1 kit
C34570	CellTrace™ CFSE Cell Proliferation Kit *for flow cytometry* *20 tests*	1 kit
C34567	CellTrace™ Yellow Cell Proliferation Kit *for flow cytometry* *180 tests*	1 kit
C34573	CellTrace™ Yellow Cell Proliferation Kit *for flow cytometry* *20 tests*	1 kit
C34564	CellTrace™ Far Red Cell Proliferation Kit *for flow cytometry* *180 tests*	1 kit
C34572	CellTrace™ Far Red Cell Proliferation Kit *for flow cytometry* *20 tests*	1 kit
Related products		
MHCD0300	Purified Mouse anti-human CD3	500 µL
PHC0026	Recombinant Human Interleukin-2	10 µg
PHC0027	Recombinant Human Interleukin-2	40 µg
S10274	SYTOX™ AADvanced™ Dead Cell Stain Kit *500 tests*	5 × 0.5 mL
S10349	SYTOX™ AADvanced™ Dead Cell Stain Kit *100 tests*	0.2 mL
S34859	SYTOX™ Red Dead Cell Stain *for flow cytometry* *1000 tests*	1 mL
S34860	SYTOX™ Green Dead Cell Stain *for flow cytometry* *1000 tests*	1 mL
11131D	DynaBeads™ CD3/CD28 T Cell Expander	2 mL
08-0022SA	OpTmizer™ T-Cell Expansion SFM	500 mL
10439-016	Fetal Bovine Serum, ES Cell-Qualified	100 mL
14190-136	Dulbecco's Phosphate-Buffered Saline (D-PBS) (1X), liquid	1000 mL

Documentation and support

These high-quality reagents and materials must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Read the Safety Data Sheet provided for each product; other regulatory considerations may apply.

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  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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Revision history: Pub. No. MAN0002595

Revision	Date	Description
E.00	11 July 2017	Add information about CellTrace™ Blue reagent, convert Spectral characteristics section into Table 2.
D.00	09 February 2016	Basis for this revision

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