Polysciences, Inc.
Chemistry beyond the ordinary

U.S. Corporate Headquarters
400 Valley Rd.
Warrington, PA 18976
1(800) 523-2575 / (215) 343-6484
1(800)343-3291 fax
info@polysciences.com

Polysciences Europe GmbH
Handelsstrasse 3
D-69214 Eppelheim, Germany
+(49) 6221-765767
+(49) 6221-764620 fax
info@polysciences.de

Polysciences Asia-Pacific, Inc.
2F-1, 207 Tunhwa N. Rd.
Taipei, Taiwan 10595
(886) 2 8712 0600
(886) 2 8712 2677 fax
info@polysciences.tw

CTC

5-Cyano-2,3-ditolyl tetrazolium chloride

BACKGROUND

The 5-cyano-2,3-ditolyl tetrazolium chloride (CTC) is a monotetrazolium redox dye which produces a fluorescent formazan (CTF) when it is chemically or biologically reduced. The CTF is deposited intracellularly. Until recently, CTC had only been employed as a cellular redox indicator of respiratory (i.e., electron transport) activity in cytochemical experiments with Ehrlich ascites tumor cells.[1-3] A recent study describes the first application of CTC for microscopic visualization of actively respiring bacteria in native and nutrient-amended environmental samples and in bacterial biofilms formed on microscope slides.[4] This compound acts similar to INT as a vital redox dye\but is more easily detected intracellularly because of its bright red fluorescence when illuminated by long-wave UV light (>350nm). Moreover, the fluorescent nature of the compound greatly facilitates its use in studying actively respiring bacteria captured on dark membrane filters or bacteria in biofilms associated with optically opaque surfaces.

CELLULAR REDOX APPLICATIONS

GENERAL

Redox dyes have the ability to change color depending on their oxidative state. To be useful in both microscopy and flow cytometric studies a redox dye would have to be fluorescent. Ideally these dyes would be water soluble in their oxidized state and only fluorescent in the reduced state. For cell isolation or localization the reduced form should be water insoluble. CTC has met these criteria and the formazan is intensely fluorescent.[1-3]

The CTC formazan is readily soluble in many polar organic solvents but is fluorescent only in the solid form, not in solution. The most useful solvents for CTC are alcohols. Ethanol has been used frequently to elute the CTC formazan, but dilute ethanol solutions (<50% ethanol) result in poor solubility. A working solution of 1 mM CTC to 2 x 106 cells has been reported by Severin, et al.[1-3]

CTC and the reduced formazan have ideal spectral properties. The unreduced CTC does not absorb light above 400nm, while the excitation max of the formazan is 450nm. The emission max of the formazan is 630nm.

FLOW CYTOMETRY

Isolation of the CTC formazan within the cell and its non-water soluble nature make it an ideal cell redox marker. Unbound CTC would not be fluorescent and reduced CTC would not be soluble so that separation of cell bound formazan from intracellular formazan or unreacted dye can be accomplished. The spectral characteristics of the formazan allow it to be excited with a 480nm laser and be detected in the red region supply wavelengths.

LIMITATIONS IN QUANTITATIVE REDOX ASSAYS

When designing assays to measure the redox potential of cells by measuring the amount of formazan produced, the insoluble formazan of the CTC may not be an advantage. In this case the insoluble formazan has to be dissolved in a solvent so that its concentration can be measured spectrophotometrically. This additional step can complicate the assay and lengthen the time of the assay. For this kind of quantitative assay we suggest the tetrazolium, XTT, which reduces to a soluble form.

STAINING PROCEDURES

The final concentration of CTC employed is 4.0 mM, although concentrations from 108 mM will work. The staining incubation times range from 30 minutes to 5 hours depending on the nature of the sample. Different water samples or microorganisms may require higher or lower concentrations and difference staining times.

WORKING SOLUTIONS

The contents of one vial of the compound (100mg) is dissolved in 6.6ml of double distilled water or ultrapure water for a final concentration of 50 mM.

STAINING OF CELLS IN SUSPENSION

1. 200 microliters of the working solution are added to a test tube containing 2ml of sample (i.e., cell suspension or environmental water samples), and incubate for one hour (axenic cell suspensions) or 4 hours (environmental water samples) at 28°C with agitation (200 rpm). Carbon supplementation (with R2A medium or glucose) may be required to maximize the number of bacteria that could actively reduce CTC in the selected time frame.
2. Stained bacteria are captured by microfiltration through a 0.2µm-pore-size black polycarbonate membrane filter. Filters are air-dried and mounted with low-fluorescence immersion oil on glass microscope slides.

STAINING OF ADHERENT CELLS

1. Cells adhered to glass or plastic microscope slides are washed using phosphate (or other suitable) buffer to remove non-adherent or loosely-attached bacteria, and then stained by dipping the slides in a 4.0 mM solution of CTC in a staining jar for one hour with carbon supplementation.
2. After incubation, the slides are rinsed in buffer and air-dried.

MICROSCOPIC EXAMINATION

For optimal viewing of red fluorescence in CTC-treated preparations, use UV irradiation with an optical filter combination consisting of a blue 420nm excitation filter (Olympus model BP490) with a 590nm barrier filter (Olympus model O590).

STORAGE AND HANDLING

Store at 4°C. CTC is an irritant and is toxic by ingestion. Wear rubber gloves and safety goggles. Handle the product with the care required by good laboratory practice. Mix waste with a combustible carrier and burn in a suitably equipped chemical incinerator. Disposal must comply with Federal, state and local regulations.

REFERENCES

1. Severin, E., J. et al, Fluorimetric assay of redox activity in cells. Anal. Chim. Acta 170:341-346 (1985).
2. Stellmach, J. Fluorescent redox dyes. I. Production of fluorescent formazan by unstimulated and phorbol ester or digitonin-stimulated Ehrlich ascites tumor cells. Histochem. J. 80:137-143 (1984).
3. Stellmach, J., et al, A fluorescent redox dye: influence of several substrates and electron carriers on the tetrazolium salt-formazan reaction of Ehrlich ascites tumor cells. Histochem. J. 19:21-26 (1987).
4. Rodriguez, G.G., et al, Use of fluorescent redox probe for direct visualization of actively respiring bacteria. Appl. Environ. Microbiol. 58(6):1801-1808 (1992).
5. Pyle, BH. et al, Appl Environ Microbiol, 65(5) 1966-72 (1999).
6. Kawai M. et al, J Appl Microbiol, 86(3) 496-504 (1999).
7. Suller MT. et al, Cytometry, 35(3) 235-41 (1999).
8. Miskin I. et al, Microbiology, 144(9) 2427-39 (1998).
9. Bremer PJ. et al, J Appl Microbiol, 85(3) 545-53 (1998).
10. Cellini L. et al, APMIS, 106(5) 571-9 (1998).
11. Severin E. et al, J Histochem Cytochem, 46(6) 761-5 (1998).
12. Cappelier JM. et al, Vet Res, 28(6) 547-55 (1997).
13. Severin E. et al, Analytica Chimica Acta, 170: 341-346 (1985).

ORDERING INFORMATION

Cat. #	Description	Size
19292	CTC (5-cyano-2,3-ditolyl tetrazolium chloride)	100mg 1g
19661	XTT	100mg 500mg
Other Tetrazolium Salts:		
00630	INT (p-Iodonitrotetrazolium violet)	1g
01165	TCNBT (Thiocarbamyl-nitroblue tetrazolium chloride)	1g

TO ORDER

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

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

In Asia Call: (886) 2 8712 0600
In Asia Fax: (886) 2 8712 2677

Order online anytime at **www.polysciences.com**