

Product Information

Cholera Toxin Subunit B CF® Dye Conjugates

Unit Size: 100 ug

See [product page](#) for a full list of product names and catalog numbers.

Storage and Handling

Store at -20°C upon arrival and protect from light. Product is stable for at least six months from date of receipt when stored as recommended.

Product Description

Cholera toxin is the symptom-causing toxin produced by the bacteria *Vibrio cholerae* during cholera infection. The toxin is composed of a hexamer of subunits A and B. Subunit A is the toxic enzymatic subunit present in one copy per toxin. Cholera toxin subunit B (CTB) is the receptor binding subunit that is found as a pentamer in each toxin and is non-toxic, making it useful for cell biological studies. These conjugates are made from purified recombinant Cholera Toxin B subunit produced in *E. coli* and are completely free of the toxic A subunit.

CTB has been used as a neuronal tracer and has also been shown to bind to GM1 gangliosides that are found in lipid rafts on the surface of mammalian cells. Therefore, fluorescently labeled conjugates of CTB have been used as lipid raft markers and endocytic tracers for live imaging or on fixed cells. Please note that CTB staining can show heterogeneity in cultured cells such as HeLa cells (1).

Biotium's Cholera Toxin Subunit B Conjugates are labeled with a selection of our CF® Dyes, a series of next-generation fluorescent dyes developed at Biotium to have combined advantages in brightness, photostability, and water solubility compared to other fluorescent dyes.

References

- 1) J. Cell Sci 117, 1421-1430 (2004).

Experimental Protocols

The following are protocols for labeling cultured cells with CTB. For neuronal tracing studies, please refer to the primary literature to find the appropriate method for a given application.

Materials required but not provided

- Phosphate-buffered saline (PBS)
- Bovine serum albumin (BSA) (Cat. No. 22013)
- Hank's Balanced Salt Solution (HBSS)
- 4% Paraformaldehyde in PBS (Cat. No. 22023)
- Hoechst dye (Cat. No. 40046) (Optional)

Reconstitution

Reconstitute CF® Dye Cholera Toxin Subunit B Conjugate in water or 1X PBS to a concentration of 1 mg/ml.

Solutions can be stored at 4°C for up to three months. For storage at 4°C, 2 mM sodium azide can be added if it is compatible with your application. Alternatively, solutions can be aliquoted and stored protected from light at -20°C for up to six months. Avoid freeze-thaw cycles if storing aliquots at -20°C.

Surface labeling on live cells

1. Wash the cells once with 1X HBSS + 0.5% BSA prechilled to 4°C.
2. Dilute the reconstituted Cholera Toxin Subunit B CF® Dye Conjugate in prechilled 1X HBSS + 0.5% BSA to a final concentration of 400 ng/mL to 1 ug/mL.
3. Remove the buffer from the cells and add the buffer with diluted conjugate. Incubate cells at 4°C for 30 minutes, protected from light.
4. Wash cells three times in prechilled 1X HBSS + 0.5% BSA.
5. Fix the cells in 4% paraformaldehyde in 1X PBS for 15 minutes at 4°C, protected from light.

Note: Hoechst or a similar stain may be used as a nuclear counterstain at 1 ug/mL. If cells will be permeabilized for other immunostaining, an antibody against cholera toxin subunit B may help in optimal preservation of the lipid raft domains and should be incubated with the cells prior to permeabilization.

6. Wash cells twice with 1X PBS and process samples for imaging or subsequent immunostaining.

Membrane vesicle trafficking assay

1. Dilute the reconstituted Cholera Toxin Subunit B CF® Dye Conjugate in complete cell culture medium at a final concentration of 400 ng/mL to 1 ug/mL conjugate and add to cells.
2. Incubate the cells with conjugate at 37°C, protected from light, for 10 minutes to 1 hour or longer.
Note: We have not observed cytotoxicity in HeLa cells after overnight incubation with Cholera Toxin Subunit B CF® Dye Conjugates.
3. Wash the cells twice with HBSS and fix in 4°C paraformaldehyde in 1X PBS for 15 minutes at room temperature, protected from light.
4. Wash the cells twice with 1X PBS and process samples for imaging or subsequent immunostaining.

Related Products

Cat. No.	Product
30111-30114	ExoBrite™ CTB EV Staining Kits
40061	RedDot™2 Far Red Nuclear Counterstain, 200X in DMSO
41033...41040	NucSpot® Nuclear Stains
40046	Hoechst 33342, 10 mg/mL in H ₂ O
22023	Paraformaldehyde, 4% in PBS, Ready-to-Use Fixative
22013	Bovine Serum Albumin Fraction V
23001...23020	EverBrite™ Mounting Medium
23008, 23009	Drop-n-Stain EverBrite™ Mounting Medium
23003...23021	EverBrite™ Hardset Mounting Medium
23017...23022	EverBrite TrueBlack® Hardset Mounting Medium
23005	CoverGrip™ Coverslip Sealant
23023	Mini Super ^{HT} PAP Pen 2.0, ~400 uses
23024	Super ^{HT} PAP Pen 2.0, ~800 uses

Please visit www.biotium.com to view our full selection of CF® Dye bioconjugates, including secondary antibodies, anti-tag and anti-hapten antibodies, phalloidin, alpha-bungarotoxin, lectins, Annexin V, and many other innovative products for life science research.

Materials from Biotium are sold for research use only, and are not intended for food, drug, household, or cosmetic use.