

# Product Information

## CF® Dye Cholera Toxin Subunit B Conjugates

Unit Size: 100 ug

### Product List

Catalog no.	Conjugate	Ex/Em (nm)
00068	Cholera Toxin Subunit B, CF@405M	408/452
00070	Cholera Toxin Subunit B, CF@488A	490/515
00074	Cholera Toxin Subunit B, CF@532	527/558
00075	Cholera Toxin Subunit B, CF@543	541/560
00071	Cholera Toxin Subunit B, CF@568	562/583
00072	Cholera Toxin Subunit B, CF@594	593/614
00077	Cholera Toxin Subunit B, CF@633	630/650
00073	Cholera Toxin Subunit B, CF@640R	642/662
00069	Cholera Toxin Subunit B, CF@647	650/665
00078	Cholera Toxin Subunit B, CF@660R	663/682
00079	Cholera Toxin Subunit B, CF@680R	680/701

### 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. This product contains less than 0.5% Cholera Toxin Subunit A and should be handled and disposed of using universal laboratory safety precautions.

### Product Description

Cholera toxin is the symptom-causing toxin produced by the bacteria *Vibrio cholerae* during cholera infection. The toxin is composed of two subunits, A and B. Subunit A is the toxic enzymatic subunit present in one copy per toxin. Cholera toxin subunit B (CT-B) is the receptor binding subunit that is found as a pentamer in each toxin and is relatively non-toxic, making it useful for cell biological studies.

CT-B 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 CT-B have been used as lipid raft markers and endocytic tracers for live imaging or on fixed cells. Please note that CT-B 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) Pang, H. et al. (2004). *J. Cell Sci* 117, 1421-1430.

### Experimental Protocols

The following are protocols for labeling of GM1 ganglioside-positive lipid rafts in cultured cells. 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 1X PBS to a concentration of 1 mg/ml. For storage at 4°C, sodium azide can be added to a final concentration of 2 mM for storage if it is compatible with your application.

Stock solutions may be prepared in water or phosphate-buffered saline. Solutions can be aliquoted and stored protected from light at -20°C for up to six months, or store at 4°C for up to three months.

### Surface labeling on live cells

1. Wash cells once with cold 1X Hank's Balanced Salt Solution (HBSS) + 0.5% (BSA).
2. Add the reconstituted CF® Dye Cholera Toxin Subunit B Conjugate to a final concentration between 400 ng/ml and 1 ug/ml to cells.
3. Incubate cells at 4°C for 30 minutes in the dark.
4. Wash cells five times with cold 1X HBSS + 0.5% BSA.
5. Fix in 4% paraformaldehyde in 1X PBS for 15 minutes at room temperature (protected from light).
 

**Note:** Hoechst 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.

### Cell trafficking assay

1. Add the reconstituted CF® Dye Cholera Toxin Subunit B Conjugate to a final concentration between 400 ng/ml and 1 ug/ml to cells in complete medium.
2. Incubate at 37°C for 10 minutes to 1 hour in the dark.
3. Fix in 4% paraformaldehyde in 1X PBS for 15 minutes at room temperature (protected from light).
 

**Note:** CF® Dye Cholera Toxin Subunit B Conjugate does not show cytotoxicity in HeLa cells after overnight incubation, however the low level of cholera toxin A subunit present may cause cytotoxicity in other cells types or after prolonged incubation times.
4. Wash cells twice with 1X PBS and process samples for imaging or subsequent immunostaining.

## Related Products

Catalog number	Product
40061	RedDot™2 Far Red Nuclear Counterstain, 200X in DMSO
40046	Hoechst 33342, 10 mg/mL in H <sub>2</sub> 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
22005	Mini Super <sup>HT</sup> Pap Pen 2.5 mm tip, ~400 uses
22006	Super <sup>HT</sup> Pap Pen 4 mm tip, ~800 uses

Please visit [www.biotium.com](http://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.