Molecular Probes™ α-Bungarotoxin and Conjugates User Guide

Catalog Numbers B56130, B13422, B35451, B13423, B35450, B1196, T1175, B1601

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product Description

Extracted from *Bungarus multicinctus* venom, α-bungarotoxin has been used to stain acetylcholine receptors in skeletal muscle, rat myotubules, the electric organ from *Torpedo californica*, and transformed *Escherichia coli*.

Invitrogen™ offers unlabeled α-bungarotoxin and α-bungarotoxin labeled with biotin or a wide selection of fluorophores. (See Table 1)

Fluorescent α-bungarotoxin derivatives are useful for visualizing the distribution of acetylcholine receptors during neuromuscular junction development. The molecular weight of unlabeled α-bungarotoxin is about 8000 daltons. Approximately one fluorophore or one biotin is attached to each α-bungarotoxin, retaining optimal binding specificity. The labeled bungarotoxins are then chromatographically separated from unlabeled molecules to ensure maximum labeling of the product.

TheAlexa Fluor™ 488 conjugate is the preferred green fluorescent derivative because of its brightness, pH sensitivity, and much greater photostablity than fluorescein α-bungarotoxin. The far-red fluorescence of Alexa Fluor™ 647 and 680 conjugates permits three and four color experiments.

Contents and Storage

Table 1 α-Bungarotoxin and α-bungarotoxin conjugates

Material	Cat. No.	Amount[1]	Fluorescence excitation / emission	Storage
α-bungarotoxin, Alexa Fluor™ Plus 405 conjugate	B56130	100 µg	411 nm / 431 nm	Store at ≤ 20°C. Desiccate. Avoid freeze-thaw cycles Protect from light
α-bungarotoxin, Alexa Fluor™ 488 conjugate	B13422	500 µg	495 nm / 519 nm	
α-bungarotoxin, Alexa Fluor™ 555 conjugate	B35451	500 µg	555 nm / 565 nm	
α-bungarotoxin, Alexa Fluor™ 594 conjugate	B13423	500 µg	590 nm / 617 nm	
α-bungarotoxin, Alexa Fluor™ 647 conjugate	B35450	500 µg	650 nm/ 668 nm	
α-bungarotoxin, biotin-XX conjugate	B1196	500 µg	N/A	
α-bungarotoxin, tetramethylrhodamine conjugate	T1175	500 µg	554 nm / 577 nm	
α-bungarotoxin, unlabeled	B1601	1 mg (lyophilized)	N/A	

[1] Unless otherwise noted, conjugates are lyophilized from phosphate-buffered saline, PBS, pH 7.4.

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Catalog numbers that appear as links open the web pages for those products.

Item	Source
Phosphate-buffered saline, PBS, pH 7.4, 1 X 1000 mL	10010-031
Hank's balanced salt solution, HBSS, with no calcium chloride, magnesium chloride, magnesium sulfate, or phenol red, 1 x 1000 mL	14175-079
Image-iT™ Fixative Solution in PBS (4% formaldehyde, methanol-free), 1 X 20 mL	FB002
Image-iT™ Glyoxal 3% Fixative, 1 x 100 mL	I28700
Image-iT™ Paraformaldehyde 4% in 0.1 M Phosphate Buffer (Methanol free), 10 x 10 mL	I28800
Deionized wate	MLS
Sodium	MLS
ProLong™ Glass Antifade Mountant, 5 x 2 mL	P36980b
EVOS™ M7000 Imaging System, 1	AMF7000

Prepare stock solution

- Prepare a 1.0 mg/mL α-bungarotoxin stock solution in PBS.
- The stock solution may be divided into aliquots and stored at -20°C for at least a month. For short term storage, add sodium azide to a final concentration of 2 mM, and store at 2-6°C.

Note: It is recommended that the protein conjugate solution is briefly centrifuged before use and that only the supernatant is added to the experiment. This step eliminates any protein aggregates that may have formed in the solutions, and reduces nonspecific background staining.

IMPORTANT! Protect from light and avoid repeated freezing and thawing.

Staining protocol

The following is an example protocol for staining 10 µm-thick, fresh-frozen, cryosections of rat skeletal muscle with fluorescent α-bungarotoxin conjugates. This protocol may require optimization for other applications. The α-bungarotoxin conjugate staining can be performed concurrently with immunofluorescence staining.

1. Fix freshly frozen sections in 4% paraformaldehyde in PBS for 15 minutes at room temperature. Alternatively, sections can be fixed in ice-cold methanol for 5 minutes at -20°C. Rinse 3 times with PBS.
2. Permeablize sections with PBS / 0.1% Triton-X™100 for 10 minutes at room temperature. Permeablilization is not require for methanol-fixed sections.
3. Prepare a 1 µg/mL α-bungarotoxin staining solution in PBS. The conjugate can also be diluted in an immunofluorescence blocking buffer.
4. Rinse several times in PBS.
5. Mount in fluorescence antifade mounting medium and cover with a coverslip.
6. Rinse several times in PBS.
7. Mount in fluorescence antifade mounting medium and cover with a coverslip.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

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Revision	Date	Description
A.0	1 March 2023	New document for Molecular Probes™ αConjugates.