



Cyto-Tracers™
GFP fusion proteins for in vivo tracing

Cat. # CYTOxxx

User Manual

Store kit at -20°C on receipt

A limited-use label license covers this product. By use of this product, you accept the terms and conditions outlined in the Licensing and Warranty Statement contained in this user manual.

(ver. 1-070610)

Contents

I.	Introduction and Background	3
II.	Available CytoTracers	5
III.	Protocol	5
A.	Transfection of pCT-Vector into Target Cells	5
B.	Transduction of pCT-Vector into Target Cells	6
C.	Creating your own Cyto-Tracer fusion protein (optional)	6
IV.	Examples	8
A.	GFP fluorescence from Cyto-Tracers with different subcellular location-specific tags	8
B.	pCT-vectors can be either transduced or transfected into cells	9
V.	Licensing and Warranty	10

List of Components

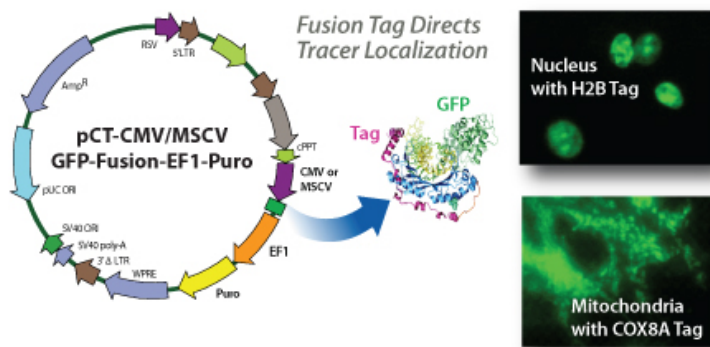
Each vial contains 10 µg plasmid DNA in TE buffer.

Vials are shipped at room temperature or on blue ice and should be stored at -20°C upon receipt. Properly stored samples are stable for 1 year from the date received.

I. Introduction and Background

Molecular trafficking is a dynamic process in eukaryotic cells. The Cyto-Tracers™ provide a means of live monitoring of molecules in real-time. SBI has created a line of lentivector-based Cyto-Tracers that utilize GFP-fusion proteins to mark cellular compartments, organelles, vesicles and structures to enable more long-term and more in-depth experimentation. Using Cyto-Tracers, cellular structures can be visualized in real-time by fluorescence microscopy without the use of chemical staining. You can also monitor the dynamic movement of a protein-of-interest in relationship to a given subcellular compartment or structure by labeling the protein-of-interest with separate fluorescent proteins.

The Cyto-Tracers are built upon an HIV-based lentiviral expression system to enable broad tropism for delivery, stable expression via integration, robust synthesis, and expression by using appropriate promoter elements. To ensure the expression of these constructs in a variety of cell types, constructs are available with two extensively-tested promoters. The Murine Stem Cell Virus promoter (MSCV) is useful for stem cells and hematopoietic cell types. The Cytomegalovirus promoter (CMV)-driven constructs can be used for other, easier to infect cell types. Cyto-Tracers can be used in transfections or packaged into virus to create stable GFP tracer cell lines in primary cells, tumor cell lines and stem cells. This allows researchers to perform preliminary plasmid-based transfection experiments before proceeding to package lentivirus particles, which allow long-term and in depth studies



Vector Components

The components of the pCT-CMV/MSCV-GFP-Fusion-EF1-Puro vectors are displayed in the table below.

Hybrid RSV-5'LTR promoter	For HIV-based vectors. Provides a high level of expression of full-length pseudoviral constructs in 293 producer cells.
cPPT, GAG, LTRs	Genetic elements necessary for the packaging, transduction, and stable integration of the viral expression construct into genomic DNA.
CMV or MSCV	Promoter that drives expression of the GFP-fusion protein in the construct. The MSCV promoter is useful for cells that are difficult to infect or transfect such as stem cells and hematopoietic cells. The CMV promoter is useful for easy-to-infect cells.
GFP-Fusion protein	This portion of the vector differs depending on the CytoTracer you have ordered.
EF1 Promoter	Drives expression of the puromycin resistance gene.

Puro	Provides puromycin resistance for creation of stable cell lines from transfection, or for selection of infected cells.
WPRE element	Enhances stability and translation of the lentivector-driven transcripts.
5' and 3' Δ -LTR	Self-inactivating LTR that enables integration into the host genome.
pUC origin	Ensures high copy replication and maintenance of the plasmid in <i>E.coli</i> cells.
Ampicillin resistance	Used for selection in <i>E. coli</i> cells.
SV40 origin	Provides stable propagation of the lentiviral plasmid in 293TN producer cells.
SV40 polyadenylation signal	Enables efficient termination of transcription and processing of recombinant transcripts.

GFP-Fusion Protein Characteristics

Each construct encodes a fusion protein consisting of an organelle-specific signaling peptide and the copGFP. The organelle-specific signaling peptide directs the copGFP to the appropriate subcellular location. SBI also offers a GFP untagged vector for researchers who would like to create their own tag for subcellular locations not currently available.

copGFP is a novel natural green monomeric GFP-like protein derived from copepod (*Pontellina sp.*). The copGFP has optimized human codons for a high level of expression of the fluorescent protein. CopGFP is a non-toxic, non-aggregating protein with fast protein maturation, high stability at a wide range of pH (pH4-12), and does not require any additional cofactors or substrates. The copGFP protein has very bright fluorescence that exceeds at least 1.3 times the brightness of EGFP, the widely used *Aequorea victoria* GFP mutant. The copGFP protein emits green fluorescence with the following characteristics:

excitation wavelength max= 482 nm

emission wavelength max = 502 nm

Due to its exceptional properties, copGFP is an excellent fluorescent marker that can be used instead of EGFP for monitoring molecular dynamics.

II. Available CytoTracers

Cyto-Tracer	Target Location	Peptide Tag
pCT-Mem-GFP	Plasma Membrane	Neuromodulin
pCT-InnerMem-GFP	Inner Leaflet of Membrane	Farnesylation signal
pCT-Mito-GFP	Mitochondria	Cytochrome C Oxidase VIII
pCT-ER-GFP	Endoplasmic Reticulum	ER targeting consensus
pCT-Golgi-GFP	Golgi Apparatus	Galactosyltransferase
pCT-Secretory-GFP	ER-Golgi Vesicles	Secretory consensus peptide
pCT-Lyso-GFP	Lysosome	Cathepsin B
pCT-Endo-GFP	Endosome	RhoB
pCT-Pero-GFP	Peroxisome	Peroxisomal Consensus Signal
pCT-Autophago-GFP	Autophagosome	LC3
pCT-CD63-GFP	Exosome/ Secretory	CD63 Tetraspanin
pCT-Actin-GFP	Cytoskeleton	β -actin
pCT-Tubulin-GFP	Cytoskeleton	α -tubulin
pCT-MAP4-GFP	Microtubule	Microtubule associated protein 4 (MAP4)
pCT-H2B-GFP	Nucleus	H2B
pCT-Apoptosis-Luc	Caspase3/7 activation	Cyclic inactive luciferase
pCT-GFP-BAX	Cytosol to Mitochondria	BAX
pCT-Catenin-GFP	Cytosol to Nucleus/ Cell Membrane	β -Catenin activation
pCT-CMV-PSD95-GFP	Dendrite Membranes	PSD-95
pCT-Cyto-GFP	Cytosolic Compartment	GFP-Untagged
pCT-Cyto-RFP	Cytosolic Compartment	RFP-Untagged
pCT-Cyto-GFP-Luc	GFP and Luciferase Control	GFP and Luciferase (Untagged)

III. Protocol

A. Transfection of pCT-Vector into Target Cells

The pCT vectors can be transfected into target cells for use in pilot experiments or for use in cells that are easy to transfect. We recommend using SBI's PureFection™ nanotechnology-based transfection reagent according to the protocol:

http://www.systembio.com/downloads/PureFection_protocol_ver2.pdf

B. Transduction of pCT-Vector into Target Cells

For cells that are more difficult to transfect, such as stem cells, primary cells, or hematopoietic cells, or to create stable GFP tracer cell lines, we recommend packaging the pCT-vector into virus and then transducing the target cells with the virus. For a protocol and list of necessary supplies and reagents, please refer to the Lentivector Expression Systems manual:

http://www.systembio.com/lentiviral-technology/delivery-systems/ppack/?id=product_11#product_8_tab_1_4

C. Creating your own Cyto-Tracer fusion protein (optional)

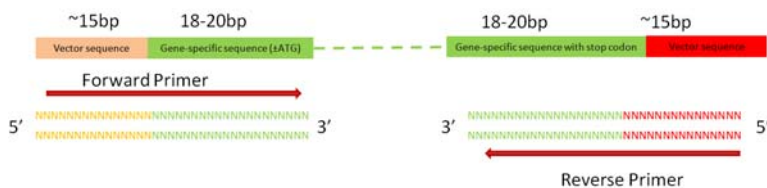
SBI offers both N- and C- terminal RFP and GFP Cyto-Tracer vectors for creating your own fusion proteins. Two strategies can be used to clone a cDNA in-frame with the RFP or GFP tag.

1. SBI's Cold Fusion Kit (Cat. # MC010A-1)
2. Use the MCS with appropriate restriction enzymes followed by sequencing of the construct.

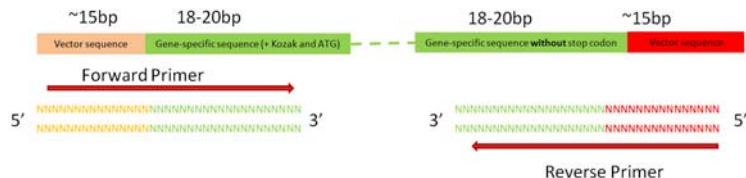
Cloning your own fusion protein into pCT with Cold Fusion

SBI's Cold Fusion kit allows you to directly clone any PCR product to any linearized expression vector, at any site. The PCR fragments can be generated by Taq DNA polymerase or other high fidelity DNA polymerases, with primers that are designed to have at least 15 bases of homology at their linear ends to where you want the product to "fuse". The linearized vector can be generated by PCR or restriction enzymes (single or double cut). The system is highly efficient, with more than 95% positive cloning rate.

To use the Cold Fusion kit with the N-terminal RFP or GFP tag, the 5' primer should contain 18-20 bases complimentary to the 5' end of your insert, plus 15 bases corresponding to the vector. You do not need to omit the ATG start site from the 5' portion of your insert. The 3' portion of the insert **MUST** contain a stop codon. The 3' primer should contain 18-20 bases complimentary to the 3' end of your insert, plus 15 bases corresponding to the vector.

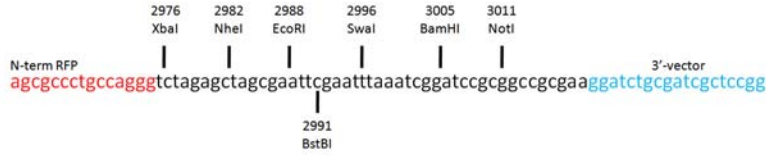


To use the Cold Fusion kit with the C-terminal RFP or GFP tag, the 5' primer should contain 18-20 bases complimentary to the 5' end of your insert, and must contain a Kozak sequence and ATG start site, plus 15 bases corresponding to the vector. The 3' end of the insert must **NOT** contain a stop codon. The 3' primer should contain 18-20 bases complimentary to the 3' end of your insert, plus 15 bases corresponding to the vector.

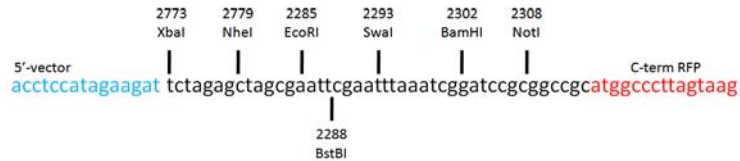


Cloning your own fusion protein into pCT using the Multiple Cloning Site

To use the MCS with the N-terminal RFP or GFP tag you do not need to omit the ATG start site from the 5' portion of your insert, but the insert should remain in-frame with the RFP or GFP tag. The 3' portion of the insert **MUST** contain a stop codon.



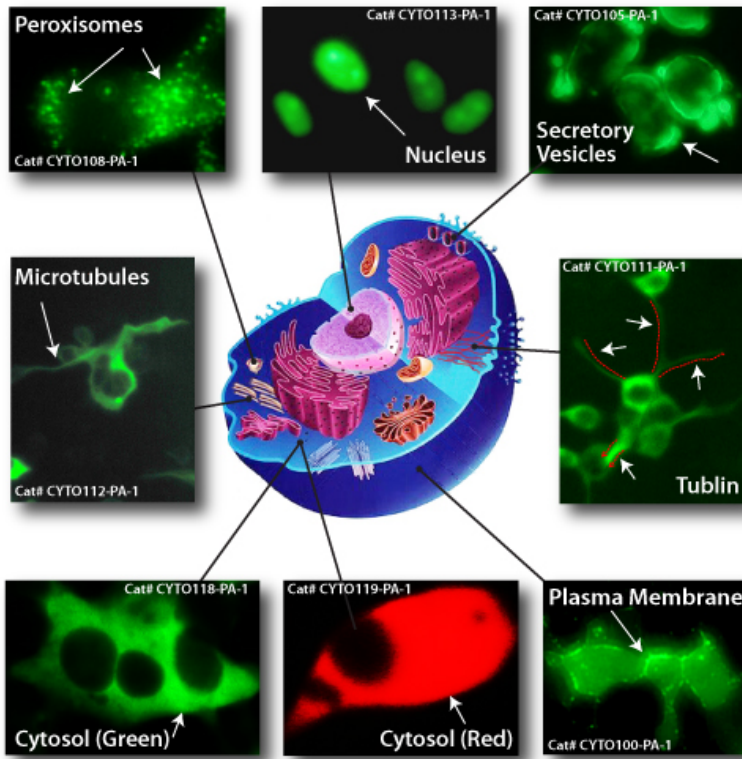
To use the MCS with the C-terminal RFP or GFP tag, your insert must contain a Kozak sequence and ATG start site. The 3' end should remain in-frame with the RFP or GFP sequence and must **NOT** contain a stop codon.



Please refer to the SBI website (<http://www.systembio.com>) for other sequences and MCS maps.

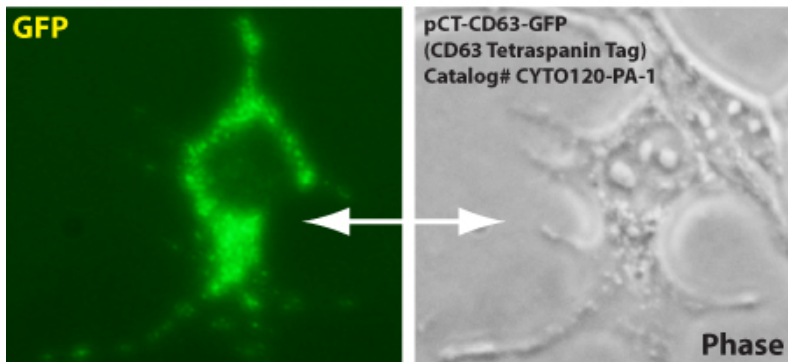
IV. Examples

A. GFP fluorescence from Cyto-Tracers with different subcellular location-specific tags

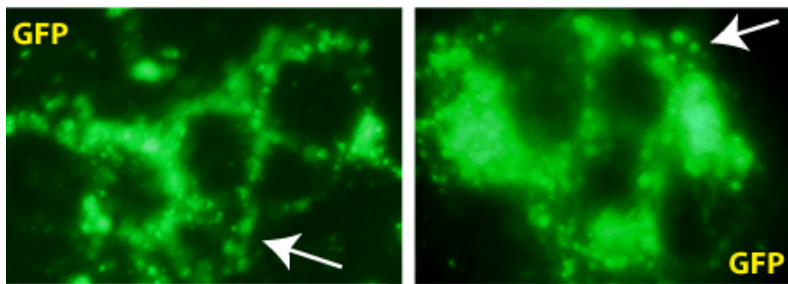


B. pCT-vectors can be either transduced or transfected into cells

Transduced 293 cells



Transfected 293 cells



V. Licensing and Warranty

Limited Use License

Use of the Cyto-Tracers (*i.e.*, the "Product") is subject to the following terms and conditions. If the terms and conditions are not acceptable, return all components of the Product to System Biosciences (SBI) within 7 calendar days. Purchase and use of any part of the Product constitutes acceptance of the above terms.

The purchaser of the Product is granted a limited license to use the Product under the following terms and conditions:

The Product shall be used by the purchaser for internal research purposes only. The Product is expressly not designed, intended, or warranted for use in humans or for therapeutic or diagnostic use.

The Product may not be resold, modified for resale, or used to manufacture commercial products without prior written consent of SBI.

This Product should be used in accordance with the NIH guidelines developed for recombinant DNA and genetic research.

HIV Vector System

This product is for non-clinical research use only. Use of this Product to produce products for resale or for any diagnostic, therapeutic, clinical, veterinary, or food purpose is prohibited. In order to obtain a license to use this Product for these commercial purposes, contact the Office of Research and Technology Ventures at the Dana-Farber Cancer Institute, Inc. in Boston, Massachusetts, USA. This Product or the use of this Product is covered by U.S. Patents Nos. 5,665,577 and 5,981,276 (and foreign equivalents) owned by the Dana-Farber Cancer Institute, Inc.

WPRE Technology

SBI has a license to sell the Product containing WPRE, under the terms described below. Any use of the WPRE outside of SBI's Product or the Products' intended use requires a license as detailed below. Before using the Product containing WPRE, please read the following license agreement. If you do not agree to be bound by its terms, contact SBI within 10 days for authorization to return the unused Product containing WPRE and to receive a full credit.

The WPRE technology is covered by patents issued to The Salk Institute for Biological Studies.

SBI grants you a non-exclusive license to use the enclosed Product containing WPRE in its entirety for its intended use. The Product containing WPRE is being transferred to you in furtherance of, and reliance on, such license. Any use of WPRE outside of SBI's Product or the Product's intended use requires a license from the Salk Institute for Biological Studies.

This license agreement is effective until terminated. You may terminate it at any time by destroying all Products containing WPRE in your control. It will also terminate automatically if you fail to comply with the terms and conditions of the license agreement. You shall, upon termination of the license agreement, destroy all Products containing WPRE in your control, and so notify SBI in writing.

This License shall be governed in its interpretation and enforcement by the laws of California.

Contact for WPRE Licensing: The Salk Institute for Biological Studies, 10010 North Torrey Pines Road, La Jolla, CA 92037; Attn: Office for Technology Management; Phone: (858) 435-4100 extension 1275; Fax: (858) 450-0509.

CMV Promoter

The CMV promoter is covered under U.S. Patents 5,168,062 and 5,385,839 and its use is permitted for research purposes only. Any other use of the CMV promoter requires a license from the University of Iowa Research Foundation, 214 Technology Innovation Center, Iowa City, IA 52242

CopGFP Control

This product contains a proprietary nucleic acid coding for a proprietary fluorescent protein(s) intended to be used for research purposes only. Any use of the proprietary nucleic acids other than for research use is strictly prohibited. **USE IN ANY OTHER APPLICATION REQUIRES A LICENSE FROM EVROGEN.** To obtain such a license, please contact Evrogen at license@evrogen.com.

SBI has pending patent applications related to the Product. For information concerning licenses for commercial use, contact SBI.

Purchase of the product does not grant any rights or license for use other than those explicitly listed in this Licensing and Warranty Statement. Use of the Product for any use other than described expressly herein may be covered by patents or subject to rights other than those mentioned. SBI disclaims any and all responsibility for injury or damage which may be caused by the failure of the buyer or any other person to use the Product in accordance with the terms and conditions outlined herein.

Limited Warranty

SBI warrants that the Product meets the specifications described in this manual. If it is proven to the satisfaction of SBI that the Product fails to meet these specifications, SBI will replace the Product or provide the purchaser with a credit. This limited warranty shall not extend to anyone other than the original purchaser of the Product. Notice of nonconforming products must be made to SBI within 30 days of receipt of the Product.

SBI's liability is expressly limited to replacement of Product or a credit limited to the actual purchase price. SBI's liability does not extend to any damages arising from use or improper use of the Product, or losses associated with the use of additional materials or reagents. This limited warranty is the sole and exclusive warranty. SBI does not provide any other warranties of any kind, expressed or implied, including the merchantability or fitness of the Product for a particular purpose.

SBI is committed to providing our customers with high-quality products. If you should have any questions or concerns about any SBI products, please contact us at (888) 266-5066.

© 2010 System Biosciences (SBI), All Rights Reserved.