Technical Data Sheet

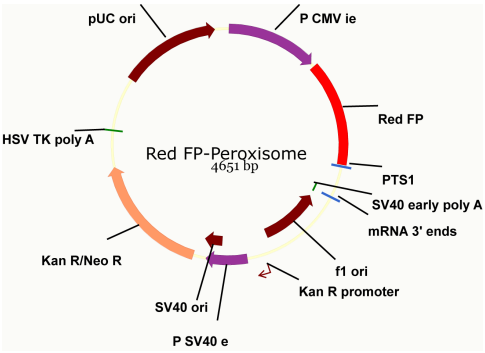
Red FP Vector - Peroxisome

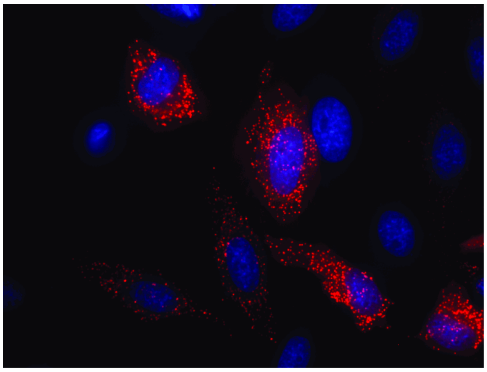
Product Information

Material Number:	**558724**
Size:	20 µg
Concentration:	0.5 mg/ml
Storage Buffer:	Aqueous buffered solution containing no preservative.

Description

BD Pharmingen™ Red FP Vector - Peroxisome is a mammalian expression vector that encodes a fusion of the red fluorescent protein (FP) from *Discosoma sp.* with the peroxisomal targeting signal 1 (PTS1), which encodes a tripeptide SKL that targets the red FP to the matrix of peroxisomes. The PTS1 sequence is fused to the 3'-end of red FP. In order to increase the translation efficiency in mammalian cells, a Kozak consensus translation initiation site has been introduced to the 5'-end of the red FP open reading frame, and its sequence has been optimized with human codons. The red FP fusion is expressed under the control of the immediate early promoter of cytomegalovirus (P CMV ie), and its sequence is followed by downstream simian virus 40 (SV40) polyadenylation signals. The vector contains a neomycin resistance gene controlled by the SV40 promoter (P SV40 e) that allows selection of stably transfected eukaryotic cells using G418. An additional bacterial (Kan R) promoter drives the expression of the same gene encoding for kanamycin resistance in *E. coli*.





LEFT: ***Map of BD Pharmingen™ Red FP Vector - Peroxisome.*** *The sequence of the entire coding region of the fluorescent protein fusion was verified by DNA sequencing, and the vector sequence can be found on our Bioimaging Certified Reagents web page, http://www.bdbiosciences.com/features/products/display_product.php?keyID=389.*

RIGHT: ***Representative merged 40x confocal image of HeLa cells stably expressing BD Pharmingen™ Red FP Vector - Peroxisome.*** *The cells were transfected and the stable population was selected according to the Recommended Assay Procedure. The transfected cells were seeded at 10,000 cells per well in a 96-well imaging plate (Cat. No. 353219), cultured overnight, washed with Hanks' Balanced Salt Solution (HBSS), and incubated in HBSS with 2 µg/ml Hoechst 33342. The cells were imaged on a BD Pathway™ 855 Bioimager System. The Red FP Vector - Peroxisome signal is pseudo-colored red, and Hoechst 33342 is pseudo-colored blue.*

Preparation and Storage

Propagation in E. coli:

- E. coli replication origin: pUC19
- Copy number: ~500
- Selectable marker: kanamycin (50 mg/ml).
- f1 origin for single-stranded DNA production
- Suitable host strains: DH5a, HB101, and other general purpose strains. Single-stranded DNA production requires a host such as JM109 or XL1-Blue that contains an F plasmid.
- Plasmid incompatibility group: pMB1/ColE1

Quality Control: For verification, the vector/insert region of each vector lot is checked by DNA sequencing, and diagnostic restriction enzyme tests are performed. In addition, each lot must have a 260/280 absorbance ratio >1.7, >90% supercoiled DNA, and endotoxin level <0.1 EU/µg.

Store undiluted at -20°C.

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Application Notes

Application

Flow cytometry	Tested During Development
Bioimaging	Tested During Development

Recommended Assay Procedure:

Transfection methods should be optimized for individual cell lines and well formats.

Transient transfection

1. Seed ~300,000 cells per well of 6-well plates on glass coverslips and culture overnight.
2. The next day transfect cells with 1 µg plasmid DNA per well using FuGENE® 6 Transfection Reagent (Roche Applied Science) according to the manufacturer's directions.
3. Cells can generally be used in experiments 24 - 48 hours post-transfection.

Stable transfection

1. Seed ~300,000 cells per well in a 6-well plate and culture overnight.
2. The next day, transfect cells with 1 µg plasmid DNA per well using FuGENE® 6 Transfection Reagent (Roche Applied Science) according to the manufacturer's directions.
3. After 48 hours, replace medium with medium supplemented with 0.5 mg/ml G418.
4. Continue selection for approximately two weeks until colonies can be identified and isolated.
   Note: Stable populations can be sorted or single-cell cloned by flow cytometry.

Detection

BD Pharmingen™ Red FP Vector - Peroxisome can be used for the localized expression of red FP in the peroxisomes of mammalian cells. It allows the visualization of the peroxisomes in living and fixed cells using fluorescence microscopy using Rhodamine or other equivalent filter sets. Red FP has an excitation maximum at 563 nm and emission maximum at 582 nm. Recommended filters for the BD Pathway™ instruments are:

Instrument	*Excitation*	*Emission*	*Dichroic*
BD Pathway 855	548/20	570LP	Fura/TRITC
BD Pathway 435	543/22	593/40	FF562

Red FP-expressing cells may be detected by flow cytometry using 488-nm excitation and the PE detector with a 585/42 nm bandpass filter. Please refer to the instrument Users Guide for more information.

Suggested Companion Products

Catalog Number	Name	Size	Clone
353219	BD Falcon™ 96-well Imaging Plate	NA	(none)

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2. The product contained herein is covered under US patents 5,874,304, 5,786,464, and 5,795,737.

References

Gould SJ, Keller GA, Hosken N, Wilkinson J, Subramani S. A conserved tripeptide sorts proteins to peroxisomes. *J Cell Biol.* 1989; 108(5):1657-1664. (Methodology)

Gould SJ, Keller GA, Schneider M, et al. Peroxisomal protein import is conserved between yeast, plants, insects and mammals. *EMBO J.* 1990; 9(1):85-90. (Biology)

Haas J, Park EC, Seed B. Codon usage limitation in the expression of HIV-1 envelope glycoprotein. *Curr Biol.* 1996; 6(3):315-324. (Methodology)

Kozak, M. An analysis of 5'-noncoding sequences from 699 vertebrate messenger RNAs. *Nucleic Acids Res.* 1987; 15(20):8125-8148. (Methodology)

Matz MV, Fradkov AF, Labas YA, et al. Fluorescent proteins from nonbioluminescent Anthozoa species. *Nat Biotechnol.* 1999; 17(10):969-973. (Methodology)

Monosov EZ, Wenzel TJ, Luers GH, Heyman JA, Subramani S. Labeling of peroxisomes with green fluorescent protein in living P. pastoris cells. *J Histochem Cytochem.* 1996; 44(6):581-589. (Methodology)

Wiemer EA, Wenzel T, Deerinck TJ, Ellisman MH, Subramani S. Visualization of the peroxisomal compartment in living mammalian cells: dynamic behavior and association with microtubules. *J Cell Biol.* 1997; 136(1):71-80. (Methodology)

Material #: 558724 Description: Red FP Vector - Peroxisome

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