Technical Data Sheet

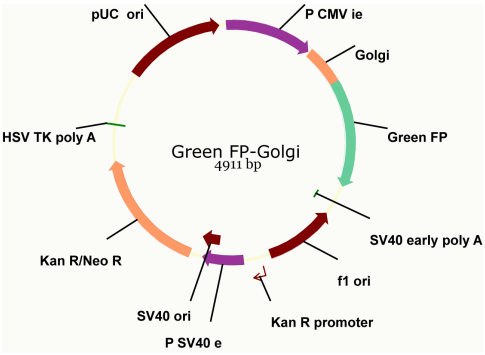
Green FP Vector - Golgi

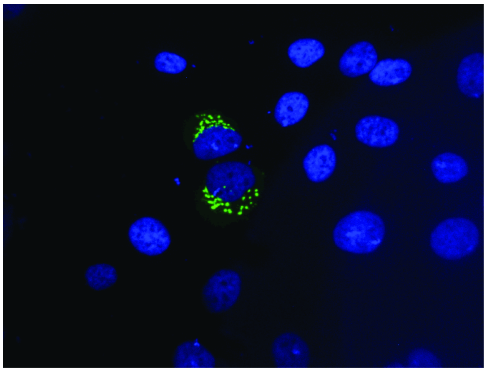
Product Information

Material Number:	**558719**
Size:	20 µg
Concentration:	0.5 mg/ml
Storage Buffer:	Aqueous buffered solution containing no preservative.

Description

BD Pharmingen™ Green FP Vector - Golgi is a mammalian expression vector that encodes a fusion of the green fluorescent protein (FP) from *Aequorea coerulescens* with the first 81 N-terminal amino acids of the human beta1,4-galactosyltransferase. The membrane-anchoring signal peptide sequence from the human beta 1,4-galactosyltransferase is fused to the 5'-end of green 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 green FP open reading frame, and its sequence has been optimized with human codons. The green 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™ Green FP Vector - Golgi.*** *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 transiently transfected with BD Pharmingen™ Green FP Vector - Golgi.*** *The cells were transfected according to the Recommended Assay Procedure and fixed with BD Cytofix™ fixation buffer (Cat. No. 554655) for 10 minutes, washed 3 times with Phosphate Buffered Saline, and mounted on slides using Vectashield mounting medium containing DAPI (Vector Laboratories). The cells were imaged on a BD Pathway™ 855 Bioimager System. The Green FP Vector - Golgi signal is pseudo-colored green, and DAPI 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™ Green FP Vector - Golgi can be used for the localized expression of green FP in the medial/trans region of the Golgi apparatus of mammalian cells. It allows the visualization of the Golgi apparatus in living and fixed cells using fluorescence microscopy using FITC, GFP, or other equivalent filter sets. Green FP has an excitation maximum at 475 nm and emission maximum at 505 nm. Recommended filters for the BD Pathway™ instruments are:

Instrument	*Excitation*	*Emission*	*Dichroic*
BD Pathway 855	488/10	515 LP	Fura/FITC
BD Pathway 435	482/35	536/40	FF506

Green FP-expressing cells may be detected by flow cytometry using 488-nm excitation and the FITC detector with a 510/20 nm bandpass filter.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554655	Fixation Buffer	100 ml	(none)
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

Gurskaya NG, Fradkov AF, Pounkova NI, et al. A colourless green fluorescent protein homologue from the non-fluorescent hydromedusa Aequorea coerulescens and its fluorescent mutants. *Biochem J.* 2003; 373(Pt 2):403-408. (Methodology)

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

Llopis J, McCaffery JM, Miyawaki A, Farquhar MG, Tsien RY. Measurement of cytosolic, mitochondrial, and Golgi pH in single living cells with green fluorescent proteins. *Proc Natl Acad Sci U S A.* 1998; 95(12):6803-6808. (Methodology)

Watzele G, Berger EG. Near identity of HeLa cell galactosyltransferase with the human placental enzyme. *Nucleic Acids Res.* 1990; 18(23):7174. (Biology)

Yamaguchi N, Fukuda MN. Golgi retention mechanism of beta-1,4-galactosyltransferase. Membrane-spanning domain-dependent homodimerization and association with alpha- and beta-tubulins. *J Biol Chem.* 1995; 270(20):12170-12176. (Biology)

Material #: 558719 Description: Green FP Vector - Golgi

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