Technical Data Sheet

Green FP Vector - Actin

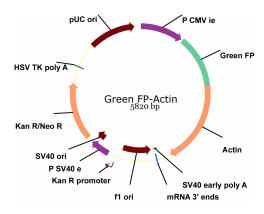
Product Information

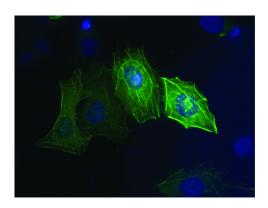
558721 **Material Number:** 20 ug 0.5 mg/ml**Concentration:**

Aqueous buffered solution containing no preservative. Storage Buffer:

Description

BD PharmingenTM Green FP Vector - Actin is a mammalian expression vector that encodes a fusion of the green fluorescent protein (FP) from Aequorea coerulescens with the the human cytoplasmic β -actin. The human β -actin sequence is fused to the 3'-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 - Actin. 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^{TI} Green FP Vector - Actin. 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 - Actin 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).
- fl 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.

BD Biosciences

bdbiosciences.com

United States 32.53.720.550 0120.8555.90 877.232.8995 888.268.5430 65.6861.0633 0800 771 7157

For country-specific contact information, visit bdbiosciences.com/how_to_order/

Conditions: The information disclosed herein is not to be construed as a recommendation to use the above product in violation of any patents. BD Biosciences will not be held responsible for patent infringement or other violations that may occur with the use of our products. Purchase does not include or carry any right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of Becton Dickinson and Company is strictly prohibited.
For Research Use Only. Not for use in diagnostic or therapeutic procedures. Not for resale.
BD, BD Logo and all other trademarks are the property of Becton, Dickinson and Company. ©2011 BD



558721 Rev. 1

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.
- 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 PharmingenTM Green FP Vector - Actin can be used for the localized expression of green FP in the actin filaments of mammalian cells. It allows the visualization of the actin filaments 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 PathwayTM 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 TM 96-well Imaging Plate	NA	(none)

Product Notices

- FuGENE, FuGENE-6, and FuGENE-HD are trademarks owned by Fugent LLC, and are protected by state, federal, and/or international trademark laws.
- 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)

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)

Ponte P, Ng SY, Engel J, Gunning P, Kedes L. Evolutionary conservation in the untranslated regions of actin mRNAs: DNA sequence of a human beta-actin cDNA. *Nucleic Acids Res.* 1984; 12(3):1687-1696. (Biology)

Westphal M, Jungbluth A, Heidecker M, et al. Microfilament dynamics during cell movement and chemotaxis monitored using a GFP-actin fusion protein. *Curr Biol.* 1997; 7(3):176-183. (Methodology)

558721 Rev. 1 Page 2 of 2

Material #: 558721 Description: Green FP Vector - Actin

>>>>>>> NOTICE <<<<<<<

Limited License for Non-Commercial Research Use Only By Purchaser

- AS A CONDITION OF THE SALE OF THE PRODUCTS AND PRIOR TO THE OPENING ANY VIALS OR CONTAINERS ENCLOSING PRODUCTS, you (the Licensee) agree to the terms and conditions set forth below.
- By opening the Product package you are indicating your agreement to the terms and conditions set forth below. If you
 do not agree to all of the terms and conditions set forth below, you should return all Product in the unopened packaging
 to BD Bioscience for a full refund.

This Limited License for Non-Commercial Research Use Only ("License Agreement") is the legal agreement between you ("Licensee"), which purchased the accompanying products and BD Biosciences ("Licensor"), for the internal research use of fluorescent protein vector technology ("Product").

I. CONTROLLING TERMS

Any terms or conditions set forth by Licensee in any writing or otherwise, including any purchase order, acknowledgement or confirmation, which are different from or in addition to those contained herein shall be null and void and shall have no binding effect whatsoever, unless agreed to in writing by Licensor.

II. PERMITTED USES THE PRODUCTS

Products are for non-commercial, research use only by the purchaser, the Licensee. LICENSEE SHALL USE THE PRODUCTS SOLELY FOR THE PURPOSE OF CONDUCTING INTERNAL, NON-COMMERCIAL RESEARCH AT ITS NOT FOR-PROFIT ORGANIZATION. LICENSEE WILL NOT SELL, TRANSFER, DISCLOSE OR OTHERWISE PROVIDE ACCESS TO THE PRODUCTS TO ANY PERSON OR ENTITY. Licensor and Licensee acknowledge that Licensee shall not have the right to authorize any third party to use third party to use or sell any Products or derivatives thereof.

III. PROHIBITED USES OF THE PRODUCT

Licensee understands that the Products, under certain circumstances, may have biological and/or chemical properties that are unpredictable and unknown at the time of transfer, that they are to be used with caution and prudence, and are not to be used for testing in or treatment of humans. The product is not to be used for drug or diagnostic purposes.

IV. LICENSE GRANT

Licensor hereby grants to Licensee a restricted, non-transferable, non-exclusive right to use the Products after the Effective Date in accordance with the terms of this License Agreement for the Term.

V. PROPERTY RIGHTS AND LIMITED WARRANTY

Title to the Product shall not transfer to Licensee and shall remain with Licensor. Licensor retains all rights not expressly granted herein and no implied licenses are granted herein. The Products are provided under one or more of the following U.S. patents 6,020,192; 5,874,304; 5,968,750; 5,795,737; 5,786,464; 7,157,565; 7183,399; 7,166,444; 7,150,979; 6,969,597, and additional patent rights pending worldwide. Licensor warrants that, at the time of shipment, the Products sold by it are free from defects in material and workmanship and conform to specifications, which accompany the Product. Notification of any claim for breach of warranty must be made within one hundred eighty (180) days of receipt of the Products. No claim shall be honored if the Licensee fails to notify Licensor within the period specified. The sole and exclusive remedy available to Licensee whether based on warranty, strict liability, contract, or otherwise, is limited to the replacement of the goods or the refund of the invoice price of the goods.

VI. COMPLIANCE WITH LAWS

Licensee shall comply with all applicable local, State, and Federal laws, rules, and regulations.

VII. DISCLAIMER OF WARRANTIES

Except as expressly provided herein, this Product and license is provided WITHOUT WARRANTIES OF ANY KIND, INCLUDING WITHOUT LIMITATION, WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, NON-INFRINGEMENT, OR ANY OTHER WARRANTY, EXPRESS OR IMPLIED.

VIII. LIMITATION OF LIABILITY

IN NO EVENT SHALL LICENSEE BE ENTITLED TO RECOVER FROM LICENSOR ANY SPECIAL, CONSEQUENTIAL, INCIDENTAL OR PUNITIVE DAMAGES, REGARDLESS OF WHETHER ADVISED OF THE POSSIBIITY OF SUCH DAMAGES.

IX. INDEMNIFICATION

Licensee agrees to indemnify, defend and hold harmless Licensor, its employees, officers and directors, from and against any and all liability, damages, losses, claims, suits, proceedings, demands, recoveries or expenses, including reasonable attorneys' fees and expenses, incurred or rendered against, that arise out of or result from the use of the Product by Licensee, except where such liability results from negligence or willful misconduct by Licensor.

X. TERM

This License Agreement shall commence upon opening of the packaging ("Effective Date") and shall continue in force so long as Products are used in accordance with the terms and conditions of this Agreement ("Term").

XI. TERMINATION

Either party may terminate this License Agreement with thirty (30) days prior written notice to the other party. The rights and obligations under Sections 1-9 and 12 shall survive any expiration or termination of this License Agreement. Upon expiration or termination of this License Agreement, Licensee shall promptly destroy all Product, its components and any progeny of the Product.

XII. ASSIGNMENT

This Agreement is not transferable or assignable by Licensee, including, without limitation upon the sale, transfer or other conveyance of the stock or assets of Licensee to another party or entity, without the prior written consent of Licensor, which consent may be withheld at Licensor's sole discretion. Licensor shall have the right to transfer and assign any or all of its rights and obligations under this License Agreement.

XIII. GOVERNING LAW

All matters affecting the interpretation, validity, and performance of this Agreement shall be governed by the laws of the State of New Jersey without regard to its conflict of law principles. Licensee hereby irrevocably consent to the personal jurisdiction of the courts of the State of New Jersey.

XIV. ENTIRE AGREEMENT

This License Agreement constitutes the entire agreement of the parties with respect to the subject matter hereof, and supersedes all prior agreements and understandings with respect to the subject matter.