



NIH AIDS Reagent Program

20301 Century Boulevard
Building 6, Suite 200
Germantown, MD 20874
USA

Phone: 240 686 4740
Fax: 301 515 4015
aidsreagent.org

DATA SHEET

Reagent: p89.6 ΔE ΔN - SF - GFP

Catalog Number: 12487

Lot Number: 140322

Release Category: C

Provided: 5 μg of dried purified DNA stabilized in DNASTable *PLUS*

Cloning Vector: pUC19
Ampicillin resistant

Cloning Site: See description for more details. A diagnostic digest with EcoRI shows an 11 kb vector band and a 3.1 kb insert band in 89.6 μEμN -SF-GFP, in comparison to 11 kb vector band and 3.8 kb insert in parental plasmid (89.6 μE-NSG).

GenBank: U39362 for wild-type 89.6
The sequence file and map for this construct is [HERE](#).

Host Strain: DH5α

Description: This construct expresses the molecular clone 89.6 (cat# 3552) bearing a both a 707 bp deletion in Env and a Nef deletion. GFP is expressed off the internal SFFV promoter. When transfected into producer cells with a separate Env expression plasmid, this construct will generate infectious virus for single round infection.

Special Characteristics: A Nef deletion was introduced into p89.6 (cat # 3552) by PCR with forward primer 89env Xho-10 (5'-CACCATATCGTTTCAGACCCT-3') and reverse primer Xho-Pme-89env (5'-TCTCGAGTTTAACTTATAGCAAAGCCCTTTCCA-3'). The reverse PCR primer contains a PmeI downstream from an XhoI site at the 5' end. p89.6 was digested with XhoI and gel purified, removing a fragment containing the 3' end of Env and 5' end of Nef. It was ligated to an XhoI digest of the PCR product, introducing a new unique PmeI site. The SFFV

ALL RECIPIENTS OF THIS MATERIAL MUST COMPLY WITH ALL APPLICABLE BIOLOGICAL, CHEMICAL, AND/OR RADIOCHEMICAL SAFETY STANDARDS INCLUDING SPECIAL PRACTICES, EQUIPMENT, FACILITIES, AND REGULATIONS. NOT FOR USE IN HUMANS.

promoter and EGFP 1500 bp insert was generated with a *NruI/PmeI* digest of pSFDNA-EGFP (see reference) and ligated into the *PmeI* site of p89.6 ΔNef. A 707 bp deletion was created in Env by religating a double blunt digest with *StuI* and *BsaBI*. The deletion was confirmed by digesting with *EcoRI* and detecting a 11 kb vector band and a 3.1 kb insert instead of a 3.8 kb insert.

The construct map can be viewed [HERE](#).

This reagent is currently being provided as dried purified DNA stabilized in DNastable *PLUS*. Please see the notice for additional information and the protocol for reconstitution of dried DNA reagents. [Dried DNA Notice](#)

Recommended Storage:	Keep the reagent at room temperature in a dry storage cabinet or in a moisture barrier bag.
Contributor:	Kathleen Collins and Ronald Collman
References:	Carter CC, Onafuwa-Nuga A, McNamara LA, Riddell J 4th, Bixby D, Savona MR, Collins KL. HIV-1 infects multipotent progenitor cells causing cell death and establishing latent cellular reservoirs. <i>Nat Med.</i> 2010 Apr;16(4):446-51. doi: 10.1038/nm.2109. Epub 2010 Mar 7. PubMed PMID: 20208541 ; PubMed Central PMCID: PMC2892382.
NOTE:	Acknowledgment for publications should read "The following reagent was obtained through the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH: Cat# 12847 p89.6 ΔE ΔN - SF - GFP from Dr. Kathleen Collins and Dr. Ronald Collman." Also include the reference cited above in any publications
Last Updated:	June 23, 2017

ALL RECIPIENTS OF THIS MATERIAL MUST COMPLY WITH ALL APPLICABLE BIOLOGICAL, CHEMICAL, AND/OR RADIOCHEMICAL SAFETY STANDARDS INCLUDING SPECIAL PRACTICES, EQUIPMENT, FACILITIES, AND REGULATIONS. NOT FOR USE IN HUMANS.