



NIH AIDS Reagent Program

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DATA SHEET

Reagent: p89.6 ΔE ΔN - SF - GFP

Catalog Number: 12487

Lot Number: 140134

Release Category: C

Provided: 1 vial of 10 μg of purified DNA at 1 mg/ml in TE buffer.

Cloning Vector: pUC19. Amp 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 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'-TCTCGAGTTTAAACTTATAGCAAAGCCCTTCCA-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 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

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.

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](#).

Recommended Storage:

-20°C or lower

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. Nat Med. 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:

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