



NIH AIDS Reagent Program

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DATA SHEET

Reagent: pBR43IeG-sykKE44nef

Catalog Number: 11374

Lot Number: 060870

Release Category: C

Provided: 3 µg of dried purified DNA stabilized in DNASTable *PLUS*

Cloning Vector: The size of the insert is about 11 kB, the vector is a truncated form of pBR322, (New England Biolabs, Ipswich, MA), and the total size of the plasmid is about 14 kB. [HERE](#) is the sequence of the pBR-NL43-IRES-eGFP-nef+ vector. All other clones differ only by their nef coding regions and the accession numbers for the different nef alleles are all listed in supplementary Table 1 of the Schindler et al. (2006) paper as well as the table linked in the reference section.

GenBank: DQ222473

Description: Nef alleles from different primate lentiviruses were cloned into an HIV-1 (NL4-3 based) proviral vector designed to co-express Nef and eGFP from a single bicistronic RNA (Schindler et al., 2003; 2006). In these constructs, nef expression is mediated by the wildtype HIV-1 LTR promoter and naturally occurring splice sites; however, cells infected with these reporter viruses co-express Nef and eGFP at correlating levels (Schindler et al., 2005). Thus, in this system the effect of Nef on the surface expression of cellular receptors or on apoptosis can be examined directly in virally infected cells. Target cells can either be infected using the wildtype X4-tropic NL4-3 Env or VSV-G pseudotyped viral particles can be used to obtain comparably high percentages of HIV-1-infected cells for flow cytometric analysis (Schindler et al., 2003; 2006).

[Plasmid Map](#)

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.

Special Characteristics:

This construct is 13753 bp including the insert.

[Sequence file lot 060870](#)

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:

Drs. Jan Münch, Michael Schindler and Frank Kirchhoff

References:

Clones 11349 and 11350:

Schindler, M., Wurfel, S., Benaroch, P., Greenough, T.C., Daniels, R., Easterbrook, P., Brenner, M., Munch, J., and Kirchhoff, F. (2003). Down-modulation of Mature MHC Class II and Up-regulation of Invariant Chain Cell Surface Expression are Well Conserved Functions of Human and Simian Immunodeficiency Virus nef alleles. *J. Virol.* **77**, 10548-10556.

Schindler, M., Munch, J., and Kirchhoff, F. (2005). HIV-1 inhibits DNA damage triggered apoptosis by a Nef-independent mechanism. *J. Virol.* **79**, 5489-5498.

Clones 11351-11380:

Schindler M, Munch J, Kutsch O, Li H, Santiago ML, Bibollet-Ruche F, Muller-Trutwin MC, Novembre FJ, Peeters M, Courgnaud V, Bailes E, Roques P, Sodora DL, Silvestri G, Sharp PM, Hahn BH, Kirchhoff F. (2006). Nef-Mediated Suppression of T Cell Activation Was Lost in a Lentiviral Lineage that Gave Rise to HIV-1. *Cell* **125**:1055-1067.

For more information on this series of IMCs, also see: Frank Kirchhoff et al. Nef Proteins from Simian Immunodeficiency Virus-Infected Chimpanzees Interact with p21-Activated Kinase 2 and Modulate Cell Surface Expression of Various Human Receptors. *J Virol.* 2004 July; **78(13)**: 6864-6874. doi: 10.1128/JVI.78.13.6864-6874.2004. PMID: PMC421647

Jan Munch et al. Nef-Mediated Enhancement of Virion Infectivity and Stimulation of Viral Replication Are Fundamental Properties of Primate Lentiviruses. *J Virol.* 2007 December; **81(24)**: 13852-13864. doi: 10.1128/JVI.00904-07. PMID: PMC2168858

The supplemental data in the following reference has a helpful table including the IMCs in this panel plus others:

Anke Heigele et al. Down-Modulation of CD8alpha-beta Is a Fundamental Activity of Primate Lentiviral Nef Proteins. *J Virol.* 2012 January; **86(1)**: 36-48. doi: 10.1128/JVI.00717-11. PMID: PMC3255914

[Table](#)

NOTE:

If intended for commercial use, please contact Dr. Frank Kirchhoff: Department of Virology, Universitätsklinikum, Albert-Einstein-Allee 11, 89081 Ulm, Germany. Phone: 49-731-50023344. Fax: 49-731-50023337. E-mail: frank.kirchhoff@medizin.uni-ulm.de.

Acknowledgment for publications should read "The following reagent was obtained through the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH: pBR-NL43-IRES-eGFP nef mutants (cat#11349-11380) from Dr. Frank Kirchhoff." Also include the references cited above in any publications.

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- pBR-NL43-IRES-eGFP-nef+ was generated by cloning an internal ribosome entry site (IRES) and the gene encoding the enhanced version of the green fluorescent protein (eGFP) into pBRNL4-3nef+ Δ 1 Δ 2 (Münch et al., 2005).
- Firstly, an HpaIHI/XbaI restriction fragment of pBRNL4-3nef+ Δ 1 Δ 2 encompassing the nef gene and the 3' LTR was cloned into pCRII-Topo (Invitrogen, Carlsbad, CA) to generate pCRII-Topo-nef+ Δ 1 Δ 2.
- Secondly, we PCR-amplified the EGFP gene of pEGFP-C1 (Clontech Laboratories, Inc), using primers introducing flanking SmaI/NheI and SmaI sites, respectively. This PCR fragment was cloned down-stream of nef into pCRII-Topo-nef+ Δ 1 Δ 2 to obtain pCRII-Topo-nef+EGFP Δ 1 Δ 2.
- Thirdly, the IRES element of pCGCG-nef-IRES-GFP was inserted between nef and eGFP using the MluI and NheI restriction sites to generate pCRII-Topo-nef+IRES-EGFP Δ 1 Δ 2.
- In the last step, the nef-IRES-eGFP-LTR fragment was cloned into pBRNL4-3nef+ Δ 1 Δ 2 to generate pBR-NL43-IRES-eGFP-nef+. Derivatives containing stop codons at positions 73 and 74 of the nef ORF either alone (nef*) or in combination with mutations in the ATG initiation codon and two in frame stop codons at positions four and five of the nef ORF (nef-) disrupting the NL4-3 nef gene were generated by standard PCR and cloning techniques.
- Splice-overlap-extension PCR was used to replace the NL4-3 nef gene with a number of heterologous HIV and SIV nef alleles (Schindler et al., 2006). Briefly, PCR fragments containing the 3' end of the NL4-3 env gene fused to the respective nef genes were cloned into pBR-NL43-IRES-eGFP-nef+ using the unique HpaI and MluI sites.

Last Updated:

February 07, 2019

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