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DATA SHEET

Reagent:	pBR43IeG-cpzTAN3nef
Catalog Number:	11358
Lot Number:	130331
Release Category:	C
Provided:	10 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:	AY536914, DQ374658
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 Sequence.

Special Characteristics:

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., Wurfl, 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. PMCID: 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. PMCID: 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. PMCID: 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.

Research Chart:

Full Name	Abbreviated Name	Accession#a	Cat#
pBR-NL43-IRES-eGFP-nef+	pBR43IeG-nef+	M19921	11349
pBR-NL43-IRES-eGFP-nef*	pBR43IeG-nef*	n/a	11350
pBR-NL43-IRES-eGFP-nef-	pBR43IeG-nef-	n/a	11351
pBR-NL43-IRES-eGFP-NA7nef	pBR43IeG-NA7nef	DQ242535	11352
pBR-NL43-IRES-eGFP-MVP8161nef	pBR43IeG-MVP8161nef	AY536905	11353
pBR-NL43-IRES-eGFP-MVP13127nef	pBR43IeG-MVP13127nef	AY536904	11354
pBR-NL43-IRES-eGFP-YBF30nef	pBR43IeG-YBF30nef	AJ006022	11355
pBR-NL43-IRES-eGFPcpzGAB2nef	pBR43IeG-cpzGAB2nef	AF382828	11356
pBR-NL43-IRES-eGFPcpzTAN1nef	pBR43IeG-cpzTAN1nef	AF447763	11357
pBR-NL43-IRES-eGFPcpzTAN3nef	pBR43IeG-cpzTAN3nef	AY536914	11358
pBR-NL43-IRES-eGFPcpzNok5nef	pBR43IeG-cpzNok5nef	AY536915	11359
pBR-NL43-IRES-eGFPgsnCM166nef	pBR43IeG-gsnCM166nef	AF468659	11360
pBR-NL43-IRES-eGFPmusCMS1085nef	pBR43IeG-musCMS1085nef	AY340700	11361
pBR-NL43-IRES-eGFPCML1nef	pBR43IeG-CML1nef	AY340701	11362
pBR-NL43-IRES-eGFPBENnef	pBR43IeG-BENnef	M30502	11363
pBR-NL43-IRES-eGFPCBL-23nef	pBR43IeG-CBL-23nef	DQ222472	11364
pBR-NL43-IRES-eGFP60415Knef	pBR43IeG-60415Knef	DQ092764	11365
pBR-NL43-IRES-eGFP310319nef	pBR43IeG-310319nef	DQ092766	11366
pBR-NL43-IRES-eGFPsmmFFm1nef	pBR43IeG-smmFFm1nef	DQ092762	11367
pBR-NL43-IRES-eGFPsmmFYr1nef	pBR43IeG-smmFYr1nef	DQ092760	11368
pBR-NL43-IRES-eGFPsmmFWr1nef	pBR43IeG-smmFWr1nef	DQ092758	11369
pBR-NL43-IRES-eGFPmac239nef	pBR43IeG-mac239nef	M33262	11370
pBR-NL43-IRES-eGFPrcmGB1nef	pBR43IeG-rcmGB1nef	AF382829	11371
pBR-NL43-IRES-eGFPdebCM5nef	pBR43IeG-debCM5nef	AY523866	11372
pBR-NL43-IRES-eGFPdebCM40nef	pBR43IeG-debCM40nef	AY523865	11373
pBR-NL43-IRES-eGFPsykKE44nef	pBR43IeG-sykKE44nef	DQ222473	11374
pBR-NL43-IRES-eGFPsykKE51nef	pBR43IeG-sykKE51nef	AY523867	11375
pBR-NL43-IRES-eGFPbluKE31nef	pBR43IeG-bluKE31nef	DQ222474	11376
pBR-NL43-IRES-eGFPSIVsunsol-36nef	pBR43IeG-SIVsunsol-36nef	DQ222476	11377
pBR-NL43-IRES-eGFPtan1nef	pBR43IeG-tan1nef	AF395566	11378
pBR-NL43-IRES-eGFPtanB87-18nef	pBR43IeG-tanB87-18nef	DQ222475	11379
pBR-NL43-IRES-eGFPsab1nef	pBR43IeG-sab1nef	U04005	11380

a- Accession numbers are for the various nef genes, not the proviral constructs

- 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+Δ1Δ2 (Münch et al., 2005).
- Firstly, an HpaIHI/XbaI restriction fragment of pBRNL4-3nef+Δ1Δ2 encompassing the nef gene and the 3`LTR was cloned into pCRII-Topo (Invitrogen, Carlsbad, CA) to generate pCRII-Topo-nef+Δ1Δ2.
- 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+Δ1Δ2 to obtain pCRII-Topo-nef+EGFPΔ1Δ2.
- 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-EGFPΔ1Δ2.
- In the last step, the nef-IRES-eGFP-LTR fragment was cloned into pBRNL4-3nef+Δ1Δ2 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: June 21, 2018