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:	SHIV 89.6P 3' Partial Molecular Clone (pSHIV-89.6 3')
Catalog Number:	4131
Lot Number:	041818
Release Category:	E
Provided:	5.78 µg of dried purified DNA stabilized in DNAstable *PLUS*
Host Strain:	Propagate in *E. coli* XL1-Blue grown at 30°C.
Description:	SHIVs are chimeric simian/human immunodeficiency viruses composed of SIVmac239 modified to include HIV-1 env and the associated auxiliary HIV-1 genes tat, vpu, and rev.
Special Characteristics:	This construct is 8924 bp including the insert. The SHIV-89.6 3' construct encodes the HIV-1 portions of the chimeric virus, cloned into the pBluescript II KS(+) vector (Stratagene). The insert size is 5.9 kb, and the plasmid size is 8.9 kb. The HIV-1 env sequences were derived from the macrophage-tropic isolate HIV-1 89.6. Infectious virus can be generated by ligating pSHIV-89.6 5' and pSHIV-89.6 3', and transfecting CEMx174 cells with the resulting construct. Contributor provided plasmid map Sequence file lot 041818 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: Dr. Joseph Sodroski

References:

Karlsson GB, Halloran M, Li J, Park IW, Gomila R, Reimann KA, Axthelm MK, Iliff SA, Letvin NL, Sodroski J. Characterization of molecularly cloned simian-human immunodeficiency viruses causing rapid CD4+ lymphocyte depletion in rhesus monkeys. *J Virol* **71**:4218-4225, 1997.

NOTE:

Acknowledgment for publications should read "The following reagent was obtained through the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH: SHIV 89.6P 3' Partial Molecular Clone (pSHIV-89.6 3') from Dr. Joseph Sodroski (cat# 4131)." Also include the reference cited above in any publications.

Recipient must not use or incorporate the reagent for commercial purposes.

Research Chart:

Transfection of CEMx174 Cells for SIV or SHIV Production

Digestion

1. Digest 5 ìg each proviral half with the appropriate restriction enzymes in a total volume of 80 µl. Remove a 5 µl aliquot and run a gel to make sure digestion has gone to completion.

   SHIV-KB9 Digest:

   Cut the 5' half clone with SphI + XhoI
   Cut the 3' half clone with SphI + NotI

   SHIV-89.6 Digest:

   Cut the 5' half clone with SphI + ClaI
   Cut the 3' half clone with SphI + AflII

2. Phenol/chloroform extract the digested DNA once. Precipitate with ethanol using standard procedures.
3. Resuspend pellets in 20 µl dH2O and set up ligations in a final volume of 50 µl, using the total 20 ìl volume of each half. Ligate for at least 3 hours at 17ºC.

Transfection

- Prepare 2M Tris buffer, pH 7.3, and 50 mM Tris buffer, pH 7.3. Filter sterilize.
- Prepare DEAE-dextran at 25 mg/ml in the 50 mM Tris buffer, pH 7.3 (0.25 g DEAE-dextran in 10 ml). Filter sterilize.
- Prepare DME/DEAE by adding 1.25 ml of the 2M Tris buffer, pH 7.3, and 0.25 ml of the 25 mg/ml DEAE-dextran solution into 48.5 ml of serum-free DMEM.
- Wash CEMx174 cells (use 5 x 106 cells for each transfection) twice in serum-free DMEM.
- Add 1.4 ml of the DME/DEAE mix to each 50 µl ligation mix. Vortex gently to mix well.
- Resuspend the cell pellet in the 1.4 ml DNA/DEAE/DMEM mix.
- Incubate for 1 hour at 37ºC.
- Centrifuge the cells. Wash once in serum-free DMEM, and once in serum-free RPMI 1640.
- Resuspend the cells in 8-10 ml RPMI 1640 containing 10% fetal bovine serum and pen-strep. Transport the cells to a containment suite, if the procedure was not already performed there.
- Monitor virus growth in the culture every two days (split the cells as needed at the same time). For SHIVs, virus is usually detected after 4-5 days, and will peak in the culture about 7-10 days. SIV is usually a little quicker.

Plasmid DNA

DNA from the plasmids containing the proviral halves can be grown in XL1-Blue bacteria. The bacteria should be grown at 30°C for better yield in DNA preparation.

Last Updated: September 19, 2018