

Centre for AIDS Reagents

Data Sheet

NAME;	pROD10 & pROD10 (modified)
REPOSITORY REFERENCE:	EVA232 & 232.1
CLONING VECTOR:	pKP59
HOST:	XL-1 Blue
CLONING SITE:	XbaI-AatII
DESCRIPTION OF CLONE:	<p>232: Complete proviral sequence of HIV-2 ROD constructed from λROD27, λROD35 and pSPE2. Flanked by 200bp of cellular DNA at the 5' end and 10bp at the 3' end. Insert 10.5Kb; Vector 2Kb. Ampicillin Resistance Marker.</p> <p>232.1: Full length infectious clone. Complete proviral sequence of HIV-2 ROD constructed from ROD27, ROD35 and pSPE5. Flanked by 200bp of cellular DNA at the 5' end and 10bp at the 3' end. Insert 10.5Kb; Vector 2Kb. SV40 origin of replication has been inserted to assist transfection into Cos cells. Ampicillin Resistance Marker.</p>
FURTHER INFORMATION:	<p>Sequencing work has identified the following mutations in the modified pROD10 - Compared to the HIV-2 ROD sequence number M15390 (NID: g1332361). There is a mutation in pSVR - a C to T single nucleotide change at position 8307 of the RNA sequence (+1 = start of RNA genome). This leads to the introduction of a stop codon and termination of the Envelope ORF at amino acid number 720. This is in the intracellular region of the transmembrane protein of envelope. The wild type version has not been checked but the pSVR version replicates well in vitro despite this mutation. Also three nucleotides changes as compared to the sequence of the HIV-2 ROD (Accession # M15390) have been found. The actual sequence of the Vpx ORF in EVA232 is:</p> <pre>ATGACAGACCCCAGAGAGACAGTACCACCAGGAAACAGCGGCGAAGAGACTATCGGAGAGGCCTTCGCCTGG CTAAACAGGACAGTAGAAGCCATAAACAGAGAAGCAGTGAATCACCTACCCCGAGAACTTATTTTCCAGGTGT GGCAGAGGTCCTGGAGATACTGGCATGATGAACAAGGGATGTCAGAAAGTTACACAAAGTATAGATATTTGTG CATAATgCAGAgAGCgGTGTACATGCATGTTAGGAAAGGGTGTACTTGCCTGGGGAGGGGACATGGGCCAGGA GGGTGGAGACCAGGGCCTCCTCCTCCTCCCCCTCCAGGTCTGGTCTAA</pre>

The changed nucleotides are indicated in lower case red letters. These changes lead to two amino acid substitutions in the corresponding Vpx protein as compared to the reference protein : I75M and K77R

PRESENTATION:

50µl DNA
EVA232 = 115 µg/ml in TE Buffer,
EVA232.1 = 545 µg/ml in TE Buffer.

CHARACTERISTICS:

Transfection into CD4+ cells results in production of infectious virus.

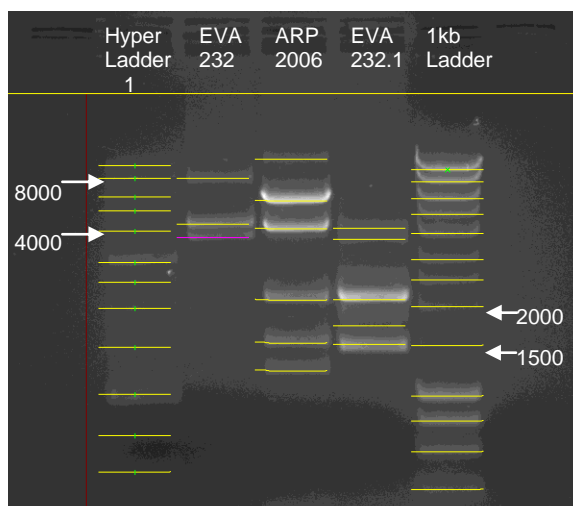
SOURCE:

Dr J-M Bechet, Institute Pasteur, Paris.
232.1 Modified by Dr AML Lever, University of Cambridge.

REFERENCE:

Clavel et al (1986) Science **233**:343
Guyader et al (1987) Nature **326**:662.

RESTRICTION PATTERNS:



Band Sizes when cut with HindIII and XbaI
EVA232 8000, 4300, 3800bp
EVA232.1 4100, 3700, 2200, 1900, 1500bp

ACKNOWLEDGEMENTS:

Publications should acknowledge the donor of the reagent and the Centre for AIDS Reagents. Suggested wording can be found on our website in the “Acknowledgement” section at:-

www.nibsc.ac.uk/spotlight/centre_for_aids_reagents.aspx

Please also ensure that you send us a copy of any papers resulting from work using reagents acquired through CFAR, this can be by e-mail or printed copy.