



pCruz 22™ Mammalian Expression Vector: sc-5041

INTRODUCTION

Santa Cruz Biotechnology offers the new pCruz series of mammalian expression vectors, a group of unique and effective vectors suitable for production of tagged recombinant fusion proteins. pCruz mammalian expression vectors are available with seven different fusion protein tags: the Myc tag, polyhistidine tag, hemagglutinin (HA) tag, Green Fluorescent Protein (GFP) tag, and the novel CruzTag series of fusion protein tags. The pCruz vectors are designed to be used together with Santa Cruz Biotechnology's fusion protein tag antibodies, which are suitable for detection and immunoprecipitation of recombinant fusion proteins encoded by the respective pCruz vectors.

Each CruzTag (09, 22, 41) is a unique twelve amino acid epitope tag that is encoded by one of the pCruz mammalian expression vectors. CruzTag-containing recombinant fusion proteins, encoded by the respective pCruz vectors, are detected with our novel series of anti-CruzTag antibodies.

SYSTEM COMPONENTS

- pCruz 22 mammalian expression vector supplied in three reading frames, A, B and C, at 20 µg each in 20 µl volume, to allow subcloning in-frame with the amino terminal fusion protein tag.
- pCruz 22 mammalian expression vector with 3 kb LacZ insert provided as a positive control.
- Antibodies are available separately for detection and purification of 22-tagged recombinant fusion proteins encoded by pCruz 22. Please inquire about catalog numbers sc-8054, sc-8601 and sc-8601-R.
- CruzTag 22 protein sequence: M R D A L D R L D R L A

DESCRIPTION OF VECTOR

The pCruz 22 mammalian expression vector features: the cytomegalovirus (CMV) mammalian expression promoter; amino terminal 22 fusion protein tag (flanked by unique Bam H1 and Eco R1 restriction sites); flexible multiple cloning site; poly A signal; Neomycin resistance gene for selection in stable mammalian expression systems; Kanamycin resistance gene for selection in *E. coli*; ori origin of replication for growth in *E. coli*. Vector map and multiple cloning site sequence are shown in Figure 1 below. Reading frames A, B and C are illustrated in Figure 2 below. Full sequence of coding region is illustrated in Figure 3 on page 2.

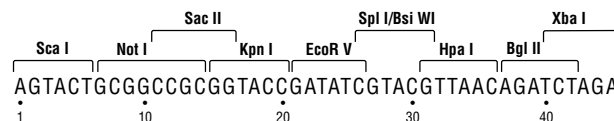
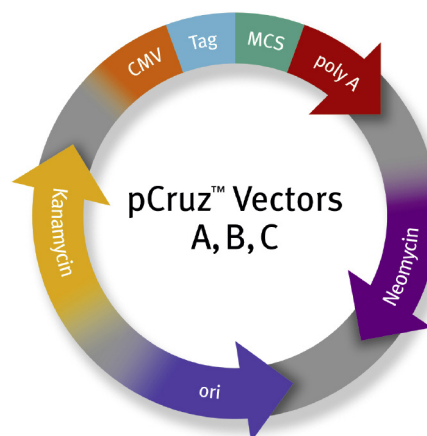


Figure 1. Vector map and multiple cloning site sequence for pCruz vectors. Unique restriction sites are indicated. Note: the Bgl II site may be methylated in some cell lines.

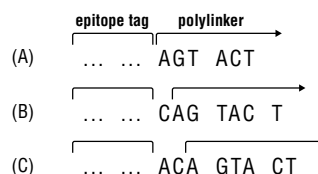


Figure 2. Reading frames A, B and C for pCruz vectors.

METHODS FOR USE

I Propagation of vectors

Prepare stock solutions of each vector and transform *E. coli* according to standard protocols. Select transformants on LB plates containing 10 µg/ml Kanamycin. Prepare glycerol stocks of transformed strains for long-term storage at -80° C.

II Cloning of target protein into pCruz vector

The target cDNA should be cloned into the multiple cloning site, using the restriction map provided to clone in-frame with the epitope tag, with no intervening in-frame stop codons. The target protein will be fused to the C-terminus of the tag protein.

III Transfection of mammalian cells with pCruz vector

Transfect into mammalian cells using a standard transfection method, such as calcium phosphate, electroporation, or liposome-based transfection. For transient transfection, allow cells to grow for 3 - 4 days, harvest, and check for protein expression. For stable transfection, allow cells to grow for 1 - 2 days (approximately to confluence) prior to Neomycin addition. Stable transformants may be selected using 100 - 800 µg/ml Neomycin, depending on the cell line.

STORAGE

Store pCruz vectors at -20° C. Spin sample briefly before pipetting. Avoid repeated freeze/thaw cycles.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

product	cat. #	fusion protein tag	amount
pCruz 09™	sc-5040	CruzTag 09™	20 µg each
pCruz 22™	sc-5041	CruzTag 22™	20 µg each
pCruz 41™	sc-5042	CruzTag 41™	20 µg each
pCruz Myc™	sc-5043	Myc Tag	20 µg each
pCruz His™	sc-5044	His-Probe	20 µg each
pCruz HA™	sc-5045	HA Tag	20 µg each
pCruz GFP™	sc-5046	EGFP Tag	20 µg each

pCruz Expression Vectors contain the bgh polyA signal, which is under license from Research Corporation Technologies, Inc. (U.S. Patent No. 5,122,458, Japan Patent No. 6-83669, Germany Patent No. P3584341.1, and EP0173552) for RESEARCH USE ONLY. RESEARCH USE does not include any of the following: any use of the bgh polyA signal in humans; any use of any material expressed or made with the use of the bgh polyA signal in humans; any transfer of the bgh polyA signal, in any form, to a third party; any use of the bgh polyA signal in connection with the expression or production of a product intended for sale or commercial use; any use of the bgh polyA signal for drug screening or drug development; any use of the bgh polyA signal for diagnostic or therapeutic purposes in humans or animals. No other rights are conveyed. Inquiry into the availability of a license for broader rights or for the use of this product for commercial purposes should be directed to David A. Wiersma, Ph.D., Research Corporation Technologies, Inc., 5210 East Williams Circle, Suite 240, Tucson, AZ 85711-4410, Tel: 1-520-748-4442, Fax: 1-520-748-0025, email: dwiersma@rctech.com.

1/1
CTA GAG AAC CCA CTG CTT ACT GGC TTA TCG AAA TTA ATA CGA CTC ACT ATA GGG AGA CCC
T7 promoter primer binding site

61/21
AAG CTT CTC GAG GCC GCC ACC ATG GGA TCC ATG CGC GAC GCC CTG GAC CGC CTG GAC CGC
M G S M R D A L D R L D R
CruzTag 22

121/41
CTG GCC GAA TTC AGT ACT GCG GCC GCG GTA CCG ATA TCG TAC GTT AAC AGA TCT AGA GGG
L A E F
MCS

181/61
CCC TAT TCT ATA GTG TCA CCT AAA TGC TAG AGC TCG CTG ATC AGC CTC GAC TGT GCC TTC
BGH reverse primer

241/81
TAG TTG CCA GCC ATC TGT TGT TTG CCC CTC CCC CGT GCC TTC CTT GAC CCT GGA AGG TGC

301/101
CAC TCC CAC TGT CCT TTC CTA ATA AAA TGA GGA AAT TGC ATC GCA TTG TCT GAG
poly A site

Figure 3. DNA sequence of pCruz 22, reading frame A. Transcription start site (T) and translation start site (ATG) are underlined. Brackets indicate sequences of the T7 promoter primer binding site, Cruz Tag 22, multiple cloning site (MCS), bovine growth hormone (BGH) reverse primer site, and poly A signal.