

pLentG-mKOSM Lentiviral Vector

CATALOG NUMBER: LTV-700

STORAGE: -80°C

QUANTITY AND CONCENTRATION: 100 µL of bacterial glycerol stock

Background

Lentiviral vectors based on the human immunodeficiency virus-1 (HIV-1) have become a promising tool for gene transfer studies. The advantageous feature of lentivirus vectors is the ability of gene transfer and integration into dividing and non-dividing cells. The pseudotyped envelope with vesicular stomatitis virus envelope G (VSV-G) protein broadens the target cell range. Lentiviral vectors have also proven to be effective in transducing brain, liver, muscle, and retina *in vivo* without toxicity or immune responses.

Induced pluripotent stem (iPS) cells can be generated from various somatic cells by the retrovirus- or lentivirus-mediated transfection of four transcription factors, namely Oct3/4, Sox2, c-Myc, and Klf4. iPS cells are indistinguishable from ES cells in morphology, proliferation, gene expression, and teratoma formation. Furthermore, when transplanted into blastocysts, iPS cells can give rise to adult chimeras, which are competent for germline transmission. However, reprogramming by viral infection of defined TFs is still inefficient (from 0.001% to 0.1 %) and requires very high transduction efficiency. Mouse embryo fibroblasts (MEFs) need at least 30% retrovirus transduction efficiency and an average of 15 different proviral copies to be reprogrammed into iPS cells. Although virus-free mouse iPS cells were recently generated by direct protein delivery, adenovirus-mediated gene delivery and DNA transfection approaches, efficiency of iPS cell generation is significantly lower (0.0001% - 0.0015%), compared with the retroviral or lentiviral infection approaches. Thus, lentivirus/retrovirus-mediated reprogramming methods are still major reprogramming approaches for generation of iPS cells, at least for basic research purposes.

pLentG-mKOSM is a simple lentiviral vector in which defined factors are in-frame fused into a single open reading frame (ORF) via self-cleaving 2A peptides and are controlled by a CMV promoter. The mouse transcription factor ORF is followed by IRES-GFP as a reporter for viral transduction. This polycistronic expression vector, when packaged into a lentivirus, can efficiently reprogram mouse or human somatic cells into iPS cells; for example, in infected MEF (GFP⁺), the reprogramming efficiency is around 1%, which is at least 10-fold higher than the reprogramming method using a pool of viruses in which each virus expresses a different stem cell factor gene. Notably, most of the iPS cells generated using the pLentG-mKOSM system contain only a single copy of the viral vector (Ref. 1).

Lentivirus Production

Lentiviral supernatant can be produced by cotransfecting 293T cells (Cat.# LTV-100) with pLentG-mKOSM and a second or third generation lentivirus packaging mix such as Cell Biolabs' ViraSafe™ Lentiviral Packaging System (Cat. # VPK-206). Supernatants can be used directly or purified/concentrated if needed. For long term storage, store supernatant at -80°C in aliquots.

Post-Packaging Considerations

1. **Concentration and purification of your lentivirus:** Because of the latent nature of lentivirus, it is imperative that your virus be highly concentrated before infecting your host cell. Also, impurities from your viral supernatant can decrease the efficiency of infection. We recommend using Cell Biolabs' ViraBind™ Lentivirus Concentration and Purification Kit (Catalog # VPK-090).
2. **Measure the titer of your lentivirus:** This is an important step to ensure consistent viral transduction into your host cell. However, QPCR or stable clone counting can take as much as 1-2 weeks to perform. Traditional p24 ELISA kits can greatly overestimate your lentiviral titer. Our advanced p24 ELISA, QuickTiter™ Lentivirus Titer Kit (Catalog # VPK-107), uses exclusive technology that eliminates free p24 from your supernatant, giving you much more accurate lentiviral titers. Results are obtained in 6-18 hours.
3. **Use transduction reagents to increase infection efficiency:** Many cells are difficult to infect with lentivirus, and without supplemental reagents transduction efficiencies can be low. Reagents such as Polybrene® can help, but are often insufficient. Cell Biolabs' proprietary reagents in our ViraDuctin™ Lentivirus Transduction Kit (Catalog # LTV-200) form a super-complex with your virus to increase transduction efficiencies by promoting virus and cell interaction.

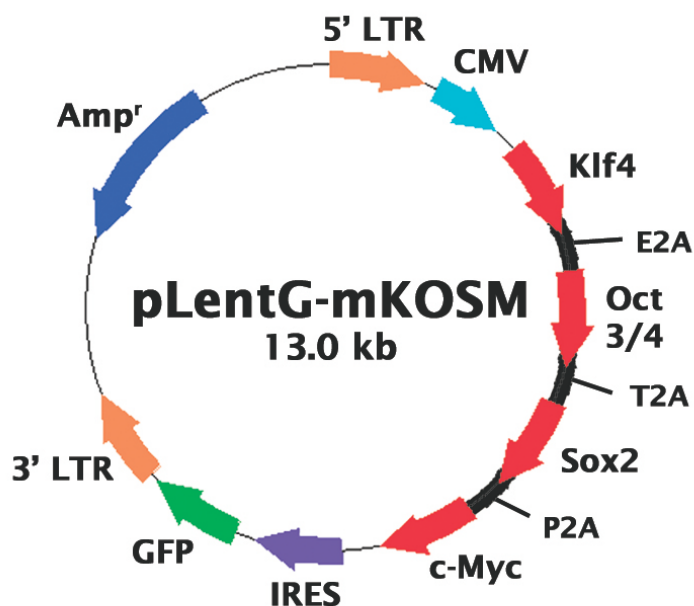


Figure 1. Schematic representation of pLentG-mKOSM lentiviral vector (13 kb). Digestion with NotI and RsrII produces 3 kb and 10 kb DNA fragments.

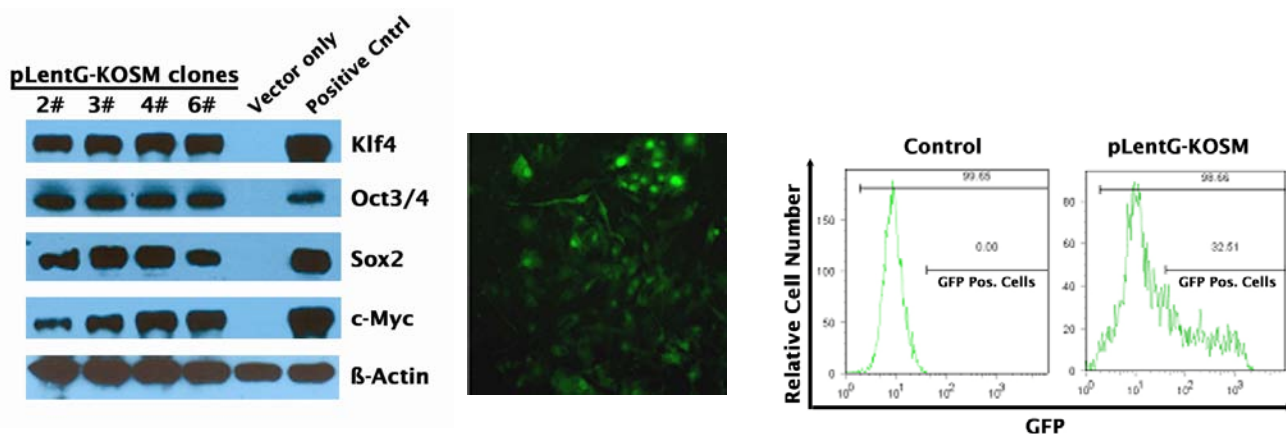


Figure 2. Expression of Stem Cell Factors and GFP. (Left) Expression of *KOSM* fusion gene in 293T cells. pLentG-mKOSM vector was transiently transfected into 293T cells. Correct processing of each factor was confirmed by western blot analysis. Expression vectors containing *KLF4*, *OCT3/4*, *SOX2*, and *c-MYC* cDNA were included as positive controls. (Middle) Representation of GFP image in MEFs. GFP fluorescence was observed 3 days after infection with lentiviruses containing the *KOSM* fusion gene. (Right) Representation of flow cytometry analysis of GFP-expressing MEFs 3 days after infection.

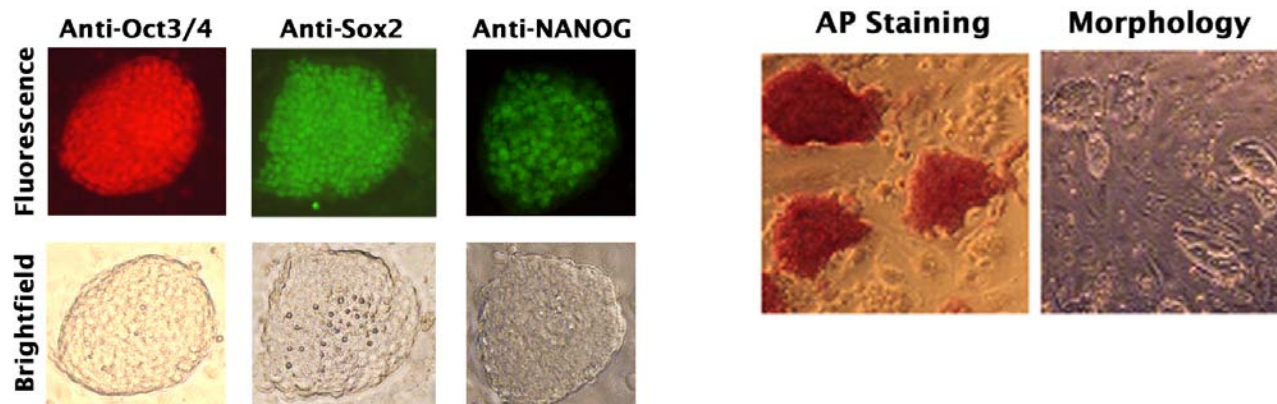


Figure 3. Characterization of induced iPS cell colonies generated from MEFs infected with the lentiviruses containing KOSM fusion gene. (Left) Staining of pluripotency markers (OCT3/4, SOX2, and NANOG) in induced cell colonies (200× magnification). (Right) AP staining (left panel, 100× magnification) and morphology (right panel, 40× magnification) in induced ES cell-like cell colonies.

Safety Consideration

Remember that you will be working with samples containing infectious virus. Follow the recommended NIH guidelines for all materials containing BSL-2 organisms. Always wear gloves, use filtered tips and work under a biosafety hood.

References

1. Shao, L, *et al.*, (2009) *Cell Research* **19**, 296-306.
2. Carey, B, *et al.*, (2009) *Proc. Natl. Acad. Sci. U. S. A.* **106**, 157-162.

3. Okita, K; Ichisaka, T; Yamanaka, S. (2007) *Nature* **448**:313–317.
4. Takahashi, K; Yamanaka, S. (2006) *Cell* **126**:663–676.
5. Takahashi, K; Tanabe, K; Ohnuki, M; Narita, M; Ichisaka, T, et al. (2007) *Cell* **131**:861–872.

License Information

The pLentG-KOSM vector system is licensed from the Maine Medical Research Institute. Purchase of this product is for research use only by the purchaser. Resale of this product to a third party is strictly prohibited.

Appendix

1. Sequence for KOSM fusion gene

Blue: Mouse Klf4; Red: Mouse Oct-3; Green: Mouse Sox2; Violet: Mouse c-Myc

Other: Self-cleaving 2A Peptides

ATGGCTGTCAGCGACGCTCTGCTCCCGTCTTCTCCACGTTTCGCGTCCGGCCCGGCGGGAAGGGAGAAGACACTGCGTCCAGCAG
 GTGCCCCGACTAACCGTTGGCGTGAGGAACCTCTCTACATGAAGCGACTTCCCCACTTCCCGGCCGCCCTACGACCTGGCGGC
 GACGGTGGCCACAGACCTGGAGAGTGGCGGAGCTGGTGCAGCTTGCAGCAGTAACAACCCGGCCCTCTAGCCCGGAGGGAGACC
 GAGGAGTTCAACGACCTCCTGGACCTAGACTTTATCCTTTCCAACCTCGCTAACCCACCAGGAATCGGTGGCCGCCACCGTGACCA
 CCTCGGCGTCACTTCATCCTCGTCTTCCCCGGCGAGCAGCGGCCCTGCCAGCGCGCCCTCCACCTGCAGCTTCAGCTATCCGAT
 CCGGGCCGGGGGTGACCCGGGCGTGGCTGCCAGCAACACAGGTGGAGGGCTCCTCTACAGCCGAGAATCTGCGCCACCTCCCACG
 GCCCCTTCAACCTGGCGGACATCAATGACGTGAGCCCCCTGGGCGGCTTCGTGGCTGAGCTCCTGCGGCCGGAGTTGGACCCAG
 TATACATTCCGCCACAGCAGCCTCAGCCGCGAGGTGGCGGGCTGATGGGCAAGTTTGTGCTGAAGGCGTCTCTGACCAACCCCTGG
 CAGCGAGTACAGCAGCCCTTCGGTTCATCAGTGTTAGCAAAGGAAGCCAGACGGCAGCCACCCCGTGGTAGTGGCGCCCTACAGC
 GGTGGCCCGCGCGCATGTGCCCAAGATTAAGCAAGAGGCGGTCCCGTCTGCACGGTCAGCCGGTCCCTAGAGGGCCATTTGA
 GCGCTGGACCCAGCTCAGCAACGGCCACCGGCCAACACACAGCACTTCCCCCTGGGGCGGCAGCTCCCCACCAGGACTACCCC
 TACACTGAGTCCCGAGGAACCTGCTGAACAGCAGGGACTGTACCCTGGCCTGCCTCTTCCCCAGGATTCCATCCCCATCCGGGG
 CCAAACCTACCTCCTTTCTGCCAGACAGATGCAGTCACAAGTCCCTCTCTCCATTATCAAGAGCTCATGCCACCGGGTTCTT
 GCCTGCCAGAGGAGCCCAAGCCAAAGAGGGGAAGAAGGTCTGTGGCCCCGGAAGAACAGCCACCCACACTTGTGACTATGCAGG
 CTGTGGCAAAACCTATACCAAGAGTTCTCATCTCAAGGCACACCTGCGAATCAGCAGGCGAGAAACCTTACCCTGTGACTGG
 GACGGCTGTGGGTGGAATTCGCCGCTCCGATGAAGTACCAGGCACTACCGCAAACACAGGGCACCAGGCCCCCTTTCAGTGCC
 AGAAGTGTGACAGGGCCTTTTCCAGGTCCGACCACTTGCCTTACACATGAAGAGGCACCTTTCGCGCCAAGCGCGGCTCCGGCCA
 GTGCACCAACTACGCCCTGCTGAAGCTGGCCGGCGACGTGGAGTCCAACCCCGGCCCGCTGGACACCTGGCTTCAGACTTCGCC
 TTCTACCCCCACAGGTGGGGGTGATGGGTGAGCAGGGCTGGAGCCGGGCTGGGTGGATCCTCGAACCTGGCTAAGCTTCCAAG
 GGCCTCAGGTGGGCTGGAATCGGACAGGCTCAGAGGTATTGGGGATCTCCCATGTCCGCCCGCATACGAGTTCTGCGGAGG
 GATGGCATACTGTGGACCTCAGGTTGGACTGGGCCTAGTCCCCCAAGTTGGCGTGGAGACTTTGCAGCCTGAGGGCCAGGCAGGA
 GCACGAGTGGAAAGCAACTCAGAGGGAACCTCCTCTGAGCCCTGTGCCGACCGCCCCAATGCCGTGAAGTTGGAGAAGGTGGAAC
 CAACTCCCGAGGAGTCCAGGACATGAAAGCCCTGCAGAAGGAGCTAGAACAGTTTCCAAGCTGCTGAAGCAGAAGAGGATCAC
 CTTGGGGTACACCCAGGCCGACGTGGGGCTCACCTGGGCGTTCTCTTTGAAAGGTGTTGAGCCAGACCACTCTGTGCTTTC
 GAGGCTTTCAGCTCAGCCTTAAGAACATGTGTAAGCTGCGGCCCTGCTGGAGAAGTGGGTGGAGGAAGCCGACAACAATGAGA
 ACCTTCAGGAGATATGCAATCGGAGACCTGGTGCAGGCCCGGAAGAGAAAGCGAACTAGCATTGAGAACCCTGTGAGGTGGAG
 TCTGGAGACCATGTTTCTGAAGTGCCGAAGCCCTCCCTACAGCAGATCACTCACATCGCCAATCAGCTTGGGCTAGAGAAGGAT
 GTGGTTCGAGTATGGTTCTGTAACCGGCGCCAGAAGGGCAAAAGATCAAGTATTGAGTATTCCAACGAGAAGAGTATGAGGCTA
 CAGGGACACCTTTCCAGGGGGGGCTGTATCCTTTCTGCCCCCAGGTCCCCACTTTGGCACCCAGGCTATGGAAGCCCCCA
 CTTACACCACTCTACTCAGTCCCTTTTCTGAGGGCGAGGCCTTTCCCTCTGTTCCCGTCACTGCTCTGGGCTCTCCCATGCAT
 TCAAAACGCGCCAAGCGCGGCTCCGGCGAGGGCAGAGGAAGTCTTCTAACATGCGGTGACGTGGAGGAGAATCCCGGCCCTTATA
 ACATGATGGAGACGGAGCTGAAGCCGCCGGGCCCGCAGCAAGCTTCGGGGGGCGGCGGCGGAGGAGGAACGCCACGGCGGCGG
 GACCGGCGGCAACCAAGAAGACAGCCGACCGCGCTCAAGAGGCCCATGAACGCCCTTCATGGTATGGTCCCGGGGGCAGCGGCGT
 AAGATGGCCAGGAGAACCCCAAGATGCACAACCTCGGAGATCAGCAAGCGCCTGGGCGCGGAGTGGAACTTTTGTCCGAGACCG
 AGAAGCGGCCGTTTCATCGACGAGGCCAAGCGGCTGCGCGCTCTGCACATGAAGGAGCACCCGGATTATAAATACCGGCCGCGGCG
 GAAAACCAAGACGCTCATGAAGAAGGATAAGTACACGCTTCCCGGAGGCTTGTGCCCCCGGCGGGAACAGCATGGCGAGCGGG
 GTTGGGGTGGGCGCGGCTGGGTGCGGGCGTGAACAGCGCATGGACAGCTACGCGCATATGAACGGCTGGAGCAACGGCAGCT
 ACAGCATGATGCAGGAGCAGCTGGGCTACCCGCGACACCCGGGCTCAACGCTCACGGCGCGGCACAGATGCAACCGATGCACCG
 CTACGACGTCAGCGCCCTGCAGTACAACCTCCATGACCAGCTCGCAGACCTACATGAACGGCTCGCCACCTACAGCATGTCTAC

TCGCAGCAGGGGACCCCCGGTATGGCGCTGGGCTCCATGGGCTCTGTGGTCAAGTCCGAGGCCAGCTCCAGCCCCCGTGGTTA
 CCTCTTCTCCCACTCCAGGGCGCCCTGCCAGGCCGGGACCTCCGGGACATGATCAGCATGTACCTCCCCGGCGCCGAGGTGCC
 GGAGCCCGCTGCGCCAGTAGACTGCACATGGCCAGCACTACCAGAGCGGCCCGGTGCCCGGCACGGCCATTAAACGGCACACTG
 CCCCCTGTCGCACATGCGCGCCAAGCGCGGCTCCGGCGCCACCAACTTCTCCCTGCTGAAGCAGGCCGGCGACGTGGAGGAGAACC
 CCGGCCCCCCCCCTCAACGTGAACTTCACCAACAGGAACATGACCTCGACTACGACTCCGTACAGCCCTATTTTCATCTGCGACGA
 GGAAGAGAATTTCTATCACCAGCAACAGCAGAGCGAGCTGCAGCCGCCCGCGCCAGTGAGGATATCTGGAAGAAATTCGAGCTG
 CTTCCACCCCGCCCTGTCCCCGAGCCGCCGCTCCGGGCTCTGCTCTCCATCCTATGTTGCGGTCGCTACGTCCTTCTCCCCAA
 GGAAGACGATGACGGCGGCGGTGGCAACTTCTCCACCGCCGATCAGCTGGAGATGATGACCGAGTTACTTGGAGGAGACATGGT
 GAACCAGAGCTTCATCTGCGATCCTGACGACGAGACCTTCATCAAGAACATCATCATCCAGGACTGTATGTGGAGCGGTTTCTCA
 GCCGCTGCCAAGCTGGTCTCGGAGAAGCTGGCCTCTACCAGGCTGCGCGCAAAGACAGCACCAGCCTGAGCCCCGCCCGCGGGC
 ACAGCGTCTGCTCCACCTCCAGCCTGTACCTGCAGGACCTACCGCCGCCGCGTCCGAGTGCAATTGACCCCTCAGTGGTCTTTCC
 CTACCCGCTCAACGACAGCAGCTCGCCCAAATCCTGTACCTCGTCCGATTCCACGGCCTTCTCTCCTTCTCGGACTCGCTGCTG
 TCCTCCGAGTCTCCCCACGGGCCAGCCCTGAGCCCTAGTGCTGCATGAGGAGACACCGCCACCACCAGCAGCGACTCTGAAG
 AAGAGCAAGAAGATGAGGAAGAAATTTGATGTGGTGTCTGTGGAGAAGAGGCCAAACCCCTGCCAAGAGGTGCGAGTGGGCTCATC
 TCCATCCCAGGGCCACAGCAAACCTCCGCACAGCCCACTGGTCTCAAGAGGTGCCACGTCTCCACTCACCAGCACAACACGCGC
 GCACCCCCCTCCACAAGGAAGGACTATCCAGCTGCCAAGAGGGCCAAGTTGGACAGTGCCAGGGTCTGAAGCAGATCAGCAACA
 ACCGCAAGTGCTCCAGCCCCAGGTCTCAGACACGGAGGAAAACGACAAGAGGCGGACACACAACGTCTTGAAACGTGAGAGGAG
 GAACGAGCTGAAGCGCAGCTTTTTTGCCTGCGTGACCAGATCCCTGAATTGAAAAACAACGAAAAGGCCCCCAAGGTAGTGATC
 CTCAAAAAAGCCACCGCTACATCCTGTCCATTCAAGCAGACGAGCACAAGCTCACCTCTGAAAAGGACTTATTGAGGAAACGAC
 GAGAACAGTTGAAACACAACTCGAACAGCTTCGAAACTCTGGTGCATAA

2. Sequence for KOSM fusion protein

Blue: Mouse Klf4; Red: Mouse Oct-3; Green: Mouse Sox2; Violet: Mouse c-Myc

Other: Self-cleaving 2A Peptides

MAVSDALLPSFSTFASGPAGREKTLRPAGAPTNRWREELSHMKRLPPLGRPYDLAATVATDLES GGAGAACSSNNPALLARRET
 EEFNDLLDLDFILSNLTHQESVAATVTTSASASSSSSPASSGPASAPSTCSFSYPIRAGGDPGVAASNTGGGLLYSRESAPPPT
 APFNLADINDVSPSGGFVAELLRPELDPVYIPPPQPPGGGLMGKFVLKASLTTPGSEYSSPSVISVSKGSPDGSHPVVPYPS
 GGPPRMCPKIKQEA VPSCTVSRSL EAHLSAGPQLSNGHRPNTHDFPLGRQLPRTTPTLSPEELLNSRDCHPGLPLPPGFHHPHG
 PNYPPFLPDQM QSQVPSLHYQELMPPGSCLPEEPKPKRGRRSWPRKRTATHTCDYAGCGKTYTKSSHLKAHLRTHTGEKPYHCDW
 DGC GWKFARSDELTRHYRKHTGHRPFQ CQKCDRAFSRSDHLALHMKRHFRAKRGSGQCTNYALLKLAGDVESNPGPAGHLASDFA
 FSPPPGGGDSAGLEPGWVDPRTWLSFQGGPPGGPGIGPGSEVLGISPCPPAYEFCGGMAYCGPQVGLGLVPQVGVETLQPEGQAG
 ARVESNSEGTSS EPCADRPNAV KLEKVEPTPEESQDMKALQKELEQFAKLLKQKRITLGYTQADVGLTLGVLFQKVFSTTTICRF
 EALQLSLKNMCKLRPLLEK WVEEADNNENLQEICKSETLVQARKRKRTSIENRVRSLETMFLKCPKPSLQQITHIANQLGLEKD
 VVRVWFCNRRQKGKRSSIEYSQREEYEATGTPFPGGAVSFPLPPGPHFGTPGYGSPHFTTLYSVPFPEGEAFPSVPVTALGSPMH
 SNRAKRGSGEGRGSLLTCGDVEENPGPYNMMETELKPPGPQQASGGGGGGGNATAAATGGNQKNSPDRVKRPMNAFMVWSRGQRR
 KMAQENPKMHNSEISKRLGAEWKLLSETEKRPFIDEAKRLRALHMKHEPDYKYRPRRKTTLMKDKYTLPGGLLAPGGNSMASG
 VGVGAGLGAGVNQRMDSYAHMNGWSNGSYMMQEQLGYPQHPGLNAHGAAQMMPMHRYDVSALQYNSMTSSQTYMNGSPTYMSY
 SQQGTGPMALGSMG SVVKSEASSSPVVTSSSHSRAPCQAGDLRDMISMYLPGA EVPEPAAPSRLHMAQHYQSGVPVGTAINGTL
 PLSHMRKRGSGATNFSLLKQAGDVEENPGPPLNVNFTNRNYDLDYDSVQPYFICDEEENFYHQQQSELQPPAPSEDIWKKFEL
 LPTPPLSPSRRSGLCSPSYVAVATSFSPREDDGGGGNFSTADQLEMMTELLGGDMV NQSFICDPDDETFIKNIIQDCMWSGFS
 AAAKL VSEKLASYQAARKDSTSLSPARGHSVCSTSSLYLQDLTAAASECIDPSVFPYPLNDSSSPKCTSSDSTAFSPSSDLL
 SSESSPRASPEPLVLHEETPPTTSSDSEEEQEDEEIDVVSVEKRQTPAKRSESGSSPSRGHSPHPLVLKRCHVSTHQHNYA
 APPSTRKDYPAAKRAKLD SGRVLKQISNNRKCSSPRSSDTEENDKRRTHNVLERQRRNELKRSFFALRDQIPELENNEKAPKVVI
 LKKATAYILSIQADEHKL TSEKDLLRKREQLKHKLEQLRNSGA-

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS' sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

This product is for RESEARCH USE ONLY; not for use in diagnostic procedures.

Contact Information

Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com

©2009-2014: Cell Biolabs, Inc. - All rights reserved. No part of these works may be reproduced in any form without permissions in writing.