upstate biotechnology
Certificate of Analysis

199 Saranac Avenue • Lake Placid, NY 12946
Technical Support: T: 800 548-7853 • F: 518 523-4513
email: techserv@upstatebiotech.com
Sales Department: T: 800 233-3991 • F: 617 890-7738
Licensing Dept.: 800 310-4659
www.upstatebiotech.com

pUSE H-*ras* (Activated) 5.4kb

Catalog # 21-103
Lot # 16447

Product Description: Transfection grade eukaryotic expression vector containing human H-*ras* (activated) under the control of the CMV promoter as illustrated.

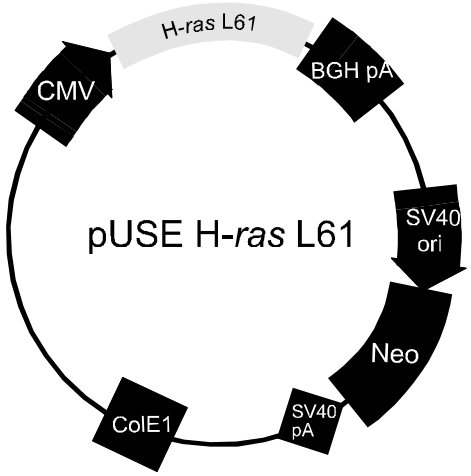
The cDNA was inserted as an Eco R1 fragment into the EcoR1 site of the pUSE(+) multiple cloning site (Catalog # 21-100).

The activating mutation is a substitution of leucine for glutamine at position 61.

Formulation: **50mg** affinity purified DNA eluted, packaged and lyophilized from 10mM Tris-HCl, pH 7.4, 1mM EDTA aseptically. Reconstitute in 100µl sterile water to 0.5mg/ml.

Storage and Stability: Store lyophilized and reconstituted DNA at -20ºC. Reconstituted DNA is stable at -20ºC for 6 months. Lyophilized DNA is stable for 1 year at -20ºC from date of shipment.

FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS



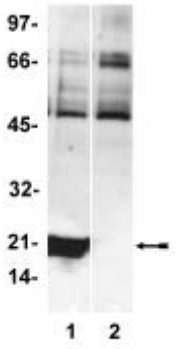
This cDNA construct is designed and intended for mammalian cell transfection only. It is **not** intended for propagation in bacteria and does **not** encode ampicillin resistance.

Quality Control Testing

Diagnostic DNA digest: List lot of DNA was cut with EcoR1, which generated the expected fragments of approximately 1 and 4.4Kb.

Purity: The OD_{260}/OD_{280} for this lot of pUSE H-*ras* (activated) is 1.76.

Expression in Transient Transfection Assay: Expression of H-ras was detected by immunoblotting of pUSE H-*ras* (activated) transfected Cos-1 cells with 2µg/ml of anti-Human ras L2 region (Catalog # 06-403).



Immunoblot Analysis
RIPA lysates of transiently transfected Cos-1 cells were resolved by electrophoresis, transferred to nitrocellulose and probed with anti-Human ras L2 region (2µg/ml). Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system. Lane 1, pUSE H-*ras* (activated) transfected cells; Lane 2 untransfected control. Arrow indicates Ras.

Background: The substitution of leucine for glutamine at position 61 of H-ras is an activating mutation that results in a five-fold increase in nucleotide exchange (GTP for GDP) in addition to decreased GTP hydrolysis. As a result, H-Ras L61 is predominantly bound to GTP, which is the activated state. Expression of H-*ras* L61 in cells therefore causes strong activation of the various effectors of Ras and transformation when stably expressed.

Reference:
Feig, L.A. and Cooper, G.M., Mol. Cell. Biol. **8**: 2472-2478, 1988.

Plasmid Information:

Multiple Cloning Site of pUSE (+) [5'? 3']:

Nhe I
Pme I
Afl II
Hind III
Asp718 I
Kpn I
BamH I
BstX I
EcoR I
EcoR V
BstX I
Not I
Xho I
Xba I
Apa I
PmeI

Schematic Map of H-*ras* cDNA :

Restriction Endonuclease Map by Position: **pUSE(+)** **Catalog # 21-100**

(Frequency < 3; Recognition sites >5 bp)
Multiple cloning site: Nhe I @ 895 nt to Pme I @ 1006 nt.

12 Bgl II
12 NcrI
31 Rrh4273I
32 Sal I
55 StySJ
80 AlwN I
161 MfeI
161 Mun I
180 Bpu10 I
208 Nru I
228 Afl III
228 Mlu I
249 Spe I
484 Nde I
590 BsaA I
590 Eco105 I
590 SnaB I
593 Eco105 I
610 Bsp19I
610 Dsa I
610 Nco I
816 EcoICR I
818 Sac I
818 SstI
876 Bsa I
876 Eco31I
895 Nhe I
899 Ace II
904 Pme I
908 Afl II
908 Bfr I
908 Bst98 I
911 Bbr I
911 EcoVIII
911 Hind III
913 Bfr I
917 Acc65 I
917 Asp718 I
921 Kpn I
922 Asp718 I
925 EcoICR I
927 Sac I
927 SstI
929 AccEBI
929 BamH I
929 BnaI
935 Spe I
948 BstX I
952 EcoR I
952 HaII
952 Rsr I
952 SsoI
957 Rsr I
961 M.SmaDam
961 Pst I
964 EcoR V
974 BstX I
979 CciNI
979 Eco52 I
979 Not I
979 Xma III
984 BsuMI
984 Sex I
985 AquI
985 Ava I
985 Ccr I
985 Eco88I
987 Sci I
989 Nli387/7 I
990 Ccr I
991 Xba I
997 Bsp120 I
997 EcoO109 I
1000 Pss I
1001 Apa I
1001 PpeI
1006 Pme I
1014 BmeTI
1015 Bcl I
1037 EcoR124 I
1217 Bbs I
1274 BavI
1274 Pvu II
1351 Rsr II
1423 NgoM I
1425 Nae I
1425 SauLPI
1525 AdeI
1528 BsaA I
1531 Dra III
1534 AdeI
1575 Drd I
1585 Bsa XI
1587 UbaD I
1684 Ace III
1726 Xmn I
1800 BstAPI
1801 ApaB I
1801 Ppu10 I
1803 BfrB I
1805 Nsi I
1822 SexA I
1872 BstAPI
1873 ApaB I
1873 Ppu10 I
1875 BfrB I
1877 Nsi I
1962 Bsp19I
1962 Dsa I
1962 Nco I
2051 BseR I
2054 Aat 1
2054 Stu I
2055 Avr II
2057 Aat 1
2076 AquI
2076 Ava I
2076 Cfr9I
2076 Eco88I
2076 PspAI
2076 Xcy I
2076 Xma I
2078 CfrJ4I
2078 Sma I
2080 Nli387/7 I
2081 Xcy I
2105 BmeTI
2106 Bcl I
2124 BsaB I
2124 Mam I
2129 Mam I
2152 BspM I
2171 Eco52 I
2171 Xma III
2264 Kas I
2265 Nar I
2265 Nun II
2266 Ehe I
2268 Bbe I
2269 Nun II
2292 Drd I
2318 Pst I
2344 Msp20 I
2347 Bal I
2347 MscI
2350 Msp20 I
2367 Fsp I
2371 BavI
2371 Pvu II
2383 Tth111 I
2490 BseMI
2498 BsrD I
2533 BspM I
2546 EcoR124 II
2569 BsaA I
2609 Sap I
2661 BscE I
2661 BseP I
2662 BssH II
2667 BscE I
2697 Bsp19I
2697 Dsa I
2697 Nco I
2765 NgoM I
2767 Nae I
2767 SauLPI
2781 Cpo I
2781 Csp I
2786 Cpo I
2819 Sap I
2947 Cbi I
2947 Csp45 I
2947 Sfu I
2951 Cbi I
2951 Sfu I
2983 BspM I
3048 Bpm I
3048 NgoM I
3050 Nae I
3050 SauLPI
3068 Gsu I
3078 EcoD XXI
3083 Gsu I
3105 Bpm I
3184 CfrA I
3185 BsaM I
3185 Bsm I
3237 Bst1107 I
3241 Rrh4273I
3242 Sal I
3440 BavI
3440 Pvu II
3479 Bsa XI
3496 StyLT III
3500 Sap I
3568 Ace III
3616 Afl III
3616 BspLU11 I
3724 Drd I
3930 Alw44I
3930 ApaL I
3935 EcoRD2
3948 EcoRD2
4032 AlwN I
4336 Bsp