Technology
Biomyx
Innovation & Convenience for Life Sciences

Biomyx Technology
10054 Mesa Ridge Court, Suite 112
San Diego, CA 92121
Phone: (858) 457-3658 FAX: (858) 457-3485
www.biomyx.net

IκB alpha ProtéGene™ Set

Catalog# I1000
Lot# On label

Materials Provided:

1. pMEV-IκBα-WT (I1000a): 20 μg in 40 μl TE (pH7.5), 0.5 mg/ml.
2. pMEV-IκBα-DN (I1000b): 20 μg in 40 μl TE (pH7.5), 0.5 mg/ml.
3. Product Information Sheets.

Note: *Individual plasmids can be ordered separately. Some plasmids are shipped as lyophilized pellet.*

Receiving and Storage:

If received in lyophilized form, add 40μl sterile DI water to the vial, dissolve thoroughly by vortex and then collect the contents by centrifuging the vials briefly in a microcentrifuge.
If received in liquid form, spin the vials briefly in a microcentrifuge to collect the contents. Store the products at 2-8^{0}C if used immediately or at –20^{0}C for extended storage.

Expression Vector:

pMEV-2HA (a): Cat# P1001a.

Affinity Tag:

N-terminal 2 x HA, a 9-aa peptide derived from influenza virus (MGYPYDVPDYAYPYDVPDYAGS…) .

Prokaryotic Selection:

The kanamycin-resistance gene (aminoglcoside 3' phospho-transferase) expression cassette in the plasmids confers Kanamycin resistance to bacteria cells. Bacterial cells transformed with the plasmids should be maintained and grown in media containing 25-50μg/ml Kanamycin (e.g. #LK-1100, Prepured LB Agar plates, Biomyx, San Diego, California).

Eukaryotic Selection:

The neomycin resistance gene, driven by SV40 early promoter, confers G418 resistance to eukaryotic cells. Stable mammalian cell lines can be selected with G418 after transfection.

Description of IκBα and Mutants

I kappa B proteins (IκBs) bind to and inhibit the NF-κB complex by trapping it in the cytoplasm (OMIM #164008). IκBs could be phosphorylated by IKKs and subsequently polyubiquitinated for degradation to allow translocation of NF-κB into the nucleus (reviewed in 1 and 2). Serines 32 and 36 in the N terminal regulatory domain of IκBα are the conserved phosphorylation sites (3). Substitution with alanines (S32A and S36A) of the phosphoacceptor sites would block the phosphorylation and degradation of IκBα by IKKs (4, 5).

Molecular Features of the Inserts:

Gene: *Homo sapiens* I kappa B alpha (IκBα)
GenBank Reference Sequence: NM_020529
5'-Cloning Site: Bam HI
5'-Junction Sequence: 5'-…tac gct gga tcc **ATG TTC CAG**-…3'
3'-Cloning Site: Xba I
3'-Junction Sequence: 5'-… gtcgactctaga **TCA TAA CGT**…-3'

hIKBA Nucleotide and Protein Sequences

(954 bps encoding 317 amino acid residues. S32, S36 and respective encoding nucleotides are in bold and underlined)

```
  1  ATGTTCCAGG CGGCCGAGCG CCCCCAGGAG TGGGCCATGG AGGGCCCCCG CGACGGGCTG
       m  f  q   a  a  e   r  p  q  e   w  a  m   e  g  p   r  d  g  l
 61  AAGAAGGAGC GGCTACTGGA CGACCGCCAC GACAGCGGCC TGGACTCCAT GAAAGACGAG
       k  k  e   r  l  l   d  d  r  h   d  S  g   l  d  S   m  k  d  e
121  GAGTACGAGC AGATGGTCAA GGAGCTGCAG GAGATCCGCC TCGAGCCGCA GGAGGTGCCG
       e  y  e   q  m  v   k  e  l  q   e  i  r   l  e  p   q  e  v  p
181  CGCGGCTCGG AGCCCTGGAA GCAGCAGCTC ACCGAGGACG GGGACTCGTT CCTGCACTTG
       r  g  s   e  p  w   k  q  q  l   t  e  d   g  d  s   f  l  h  l
241  GCCATCATCC ATGAAGAAAA GGCACTGACC ATGGAAGTGA TCCGCCAGGT GAAGGGAGAC
       a  i  i   h  e  e   k  a  l  t   m  e  v   i  r  q   v  k  g  d
301  CTGGCTTTCC TCAACTTCCA GAACAACCTG CAGCAGACTC CACTCCACTT GGCTGTGATC
       l  a  f   l  n  f   q  n  n  l   q  q  t   p  l  h   l  a  v  i
361  ACCAACCAGC CAGAAATTGC TGAGGCACTT CTGGGAGCTG GCTGTGATCC TGAGCTCCGA
       t  n  q   p  e  i   a  e  a  l   l  g  a   g  c  d   p  e  l  r
421  GACTTTCGAG GAAATACCCC CCTACACCTT GCCTGTGAGC AGGGCTGCCT GGCCAGCGTG
       d  f  r   g  n  t   p  l  h  l   a  c  e   q  g  c   l  a  s  v
481  GGAGTCCTGA CTCAGTCCTG CACCACCCCG CACCTCCACT CCATCCTGAA GGCTACCAAC
       g  v  l   t  q  s   c  t  t  p   h  l  h   s  i  l   k  a  t  n
541  TACAATGGCC ACACGTGTCT ACACTTAGCC TCTATCCATG GCTACCTGGG CATCGTGGAG
       y  n  g   h  t  c   l  h  l  a   s  i  h   g  y  l   g  i  v  e
601  CTTTTGGTGT CCTTGGGTGC TGATGTCAAT GCTCAGGAGC CCTGTAATGG CCGGACTGCC
       l  l  v   s  l  g   a  d  v  n   a  q  e   p  c  n   g  r  t  a
661  CTTCACCTCG CAGTGGACCT GCAAAATCCT GACCTGGTGT CACTCCTGTT GAAGTGTGGG
       l  h  l   a  v  d   l  q  n  p   d  l  v   s  l  l   l  k  c  g
721  GCTGATGTCA ACAGAGTTAC CTACCAGGGC TATTCTCCCT ACCAGCTCAC CTGGGGCCGC
       a  d  v   n  r  v   t  y  q  g   y  s  p   y  q  l   t  w  g  r
781  CCAAGCACCC GGATACAGCA GCAGCTGGGC CAGCTGACAC TAGAAAACCT TCAGATGCTG
       p  s  t   r  i  q   q  q  l  g   q  l  t   l  e  n   l  q  m  l
841  CCAGAGAGTG AGGATGAGGA GAGCTATGAC ACAGAGTCAG AGTTCACGGA GTTCACAGAG
       p  e  s   e  d  e   e  s  y  d   t  e  s   e  f  t   e  f  t  e
901  GACGAGCTGC CCTATGATGA CTGTGTGTTT GGAGGCCAGC GTCTGACGTT ATGA
       d  e  l   p  y  d   d  c  v  f   g  g  q   r  l  t   l  -
```

Mutations:

pMEV-IKBA-WT (I1000a): No mutation
pMEV-IKBA-DN (I1000b): S32A; S36A

References:

1. Baldwin, A. S. (1996) *Annu. Rev. Immunol.* 14: 649-681 The NF-kappa B and I kappa B proteins: new discoveries and insights
2. Verma, I. M., Stevenson, J. K., Schwarz, E. M., et al., (1995) *Genes Dev.* 9, 2723-2735. Rel/NF-kappa B/I kappa B family: intimate tales of association and dissociation
3. Karin M. (1999) *J Biol Chem.* 274(39): 27339-42The Beginning of the End: IκB Kinase (IKK) and NF-κB Activation
4. J DiDonato, F Mercurio, C Rosette et al., (1996) *Mol. Cell. Biol.*,16(4): 1295-1304 Mapping of the inducible IkappaB phosphorylation sites that signal its ubiquitination and degradation
5. Jaspers I., Samet, JM, and Reed W., (1999). Arsetite exposure of cultured airway epithelial cells activates kB-dependent interleukin-8 gene expression in the absence of nuclear factor-kB nuclear translocation. J. Biol. Chem., 274: 31025-33