

Tables

Overview of Cloned Plasmids

Name	Plasmid	Insert	Restriction Site
pZO 01	p2Bac/pFastBac-KKCK-CaM	KKCK-tagged CaM	Not I/Sal I
pZO 02	p2Bac/pFastBac-CRECC-CaM	CCRECC-tagged CaM	Not I/Sal I
pZO 04	p2Bac/pFastBac-wt-M6-KKCK-CaM ^m	wild type-M6	EcoR I/ BamH I
pZO 05	p2Bac/pFastBac-wt-M6-CCRECC-CaM ^m	wild type-M6	EcoR I/ BamH I
pZO 06	p2Bac/pFastBac-YFP-del-wt-M5-wt-CaM	YFP-deleted-wt-M5	Sph I/Spe I
pZO 07	p2Bac/pFastBac-YFP-del-wt-M6-KKCK-CaM ^m	YFP-deleted-wt-M6	BamH I/ EcoR I
pZO 08	Bluescript psK ⁺ / wt-S1	wild type-S1	NcoI /Xba I
pZO 09	Bluescript psK ⁺ / M-724	CCXXCC-tagged S1	NcoI /Xba I
pZO 10	Bluescript psK ⁺ / M-751	CCXXCC-tagged S1	NcoI /Xba I
pZO 11	pTIKL OE/ M-724	CCXXCC-tagged S1	NcoI /Xba I
pZO 12	pTIKL OE/ M-751	CCXXCC-tagged S1	NcoI /Xba I

Overview of PCR Primers

p2Bac/pFastBac-KKCK-CaM^m (pZO 1)

- **Forward Primer (Fwd 01)**
5'-ATAGCGGCCGCATGAAGAAGTGTAAGGCAGATCAACTGACAGAAGAGC-3'
- **Reverse Primer (Rev 01)**
5'-GCGTCGACTCACTTCGCTGTCATCATCTG-3'

p2Bac/pFastBac-CCRECC-CaM^m (pZO 2)

- **Forward Primer (Fwd 02)**
5'-ATAGCGGCCGCATGTGGGAGGCCGCTGCTAGGGAGGCCTGC
TGCAGGGAGTGCTGCGCTAGGGCCGCAGATCAACTGAC-3'
- **Reverse Primer (Rev 02)**
5'-GCGTCGACTCACTTCGCTGTCATCATCTG-3'

p2Bac/pFastBac- Δ YFP-wt-M5-wt-CaM (pZO 5)

- **Forward Primer (Fwd 07)**
5'- ATAGCATGCGAGATGCTACTATTGCTC -3'
- **Reverse Primer (Rev 07)**
5'- TATACTAGTTCACTCCCCGACAAGCTTCTTAAGT -3'

Bluescript psK⁺/ M-724 (pZO 09)

- **Forward Primer for M-724 (Fwd 09)**
5'- CACAAAAAGCCACCTGTTGTGTTCTCTGCTGTCTTAACATTGATCC-3'
- **Reverse Primer for M-724 (Rev 09)**
5'-GGATCAATGTTAAGACAGCAGAGAACACAACAGGTGGCTTTTTGTG-3'

Bluescript psK⁺/ M-751 (pZO 10)

- **Forward Primer for M-751(Fwd 10)**
5'-CCGTGCCGGTCAATGCTGTCGTATTTGCTGCGCTCGTGAACAACG-3'
- **Reverse Primer for M-751(Rev10)**
5'-CGTTGTTACGAGCGCAGCAAATACGACAGCATTGACCGGCACGG-3'

Sequencing Primers for p2Bac/pFastBac-KKCK-CaM^m

- **Forward Primer (Fwd 03)**
5'-GCCGGGACCTTTAATTCAACCCAACAC-3'
- **Reverse Primer (Rev 03)**
5'-AAATTTGCGGCCCGCCATGGCAGATCAACTGACAGAAGAGC-3'

Bacmid Analysis Primers

- **M13 Forward Primer (Fwd 04)**
5'-GTTTTCCCAGTCACGAC-3'
- **GenR#1 Reverse Primer (Rev 04)**
5'-ATTCATCGCGCTTGCTGCC-3'

- **pGFP#4 Forward Primer (Fwd 05)**
5'-CTTTCGGGCATGGCGGAC-3'
- **M13 Reverse primer (Rev 05)**
5'-CAGGAAACAGCTATGAC-3'

Overview of Chemicals and their Sources

Chemicals	Source
20-50 Mesh Bio-Beads SM-2	Bio-Rad
Acrylamide	Bio-Rad
ADP*VO ₄	Sigma-Aldrich
AEBSF	Sigma-Aldrich
Agarose	Sigma-Aldrich
Amoniumpersulfate (APS)	J.T. Baker
AMP*PMP	Sigma-Aldrich
Anti FLAG® M2 Affinity Resin	Sigma-Aldrich
Anti-GFP antibody	Qbiogene
Aprotinin	Sigma-Aldrich
APS	Sigma-Aldrich
ATP	Calbiochem
Bacto tryptone	Difco
Bacto yeast extract	Difco
BenchMark protein ladder	Invitrogen
Benzamidine	Sigma-Aldrich
Bicine	Sigma-Aldrich
Bis-acrylamide	Bio-Rad
Bluo-gal	Invitrogen
Boronate resin	Pierce, Rockford
Bromophenol Blue	Sigma-Aldrich
BSA	Sigma-Aldrich
Carbenicillin	Sigma-Aldrich
Cellfectin®	Invitrogen
Cloramphenicol	Sigma-Aldrich
Collodion solution	Electron Microscopy Sciences
Coomassie Brilliant Blue	Bio-Rad
Creatine phosphate	Calbiochem

Cy3 maleimide	Amersham Biosciences
Cy5 maleimide	Amersham Biosciences
DNA ladder (1 kb)	Invitrogen
DTT	Roche
EDTA	Sigma-Aldrich
EGTA	Sigma-Aldrich
Ethidium bromide	Sigma-Aldrich
FBS	Gibco
FLAG [®] peptide	Invitrogen
FM media	GibcoBRL
G418	Gibco
Gentamicin	Sigma-Aldrich
Glucose	Sigma-Aldrich
Glycerole	J.T. Baker
Guanidium hydrochloride	Fisher Scientific
Hepes	J.T. Baker
Igepal	Sigma-Aldrich
Imidazole	J.T. Baker
Iodoacetamide	Sigma-Aldrich
IPTG	Research Products International
Iso-amylacetate	Sigma-Aldrich
Kanamycin	OmniPur
Leupeptin	Sigma-Aldrich
L-glutamine	Gibco
MES	Sigma-Aldrich
MOPS	J.T. Baker
NADH	Sigma-Aldrich
N-Ethylmaleimide	Sigma-Aldrich
NeutrAvidin	Molecular Probes
Ni-NTA agarose beads	Qiagen
NTCB	Sigma-Aldrich

Penicillin	Geneva Pharmaceuticals
Pepstatin A	Sigma-Aldrich
Phosphocreatine	Calbiochem
PMSF	Sigma-Aldrich
Proteose peptone	Oxoide
Sf-900 II SFM media	Gibco
Sodiumdodecylsulfate (SDS)	J.T. Baker
Streptavidin polystyrene beads (90 nm)	Bangs Laboratories
Streptomycin	Gibco
Sucrose	Fisher Scientific
TCEP	Molecular Probes
TEMED	OmniPur
Tetracycline	Sigma-Aldrich
TFA	Sigma-Aldrich
TLCK	Sigma-Aldrich
TPCK	Sigma-Aldrich
TRITC phalloidin	Molecular Probes
Triton-X	Sigma-Aldrich
Trypan blue	Invitrogen
Urea	J.T. Baker
Yeast extract	Oxoide
β -Mercaptoethanol	Sigma-Aldrich

Overview of Enzymes and their Sources

Enzymes	Source
BamH I	New England Biolabs
Catalase	Sigma
Creatine phosphokinase	Calbiochem
DNase I	Roche Diagnostics

EcoR I	New England Biolabs
Glucose oxidase	Calbiochem
Nco I	New England Biolabs
Not I	New England Biolabs
PEP	Sigma
Pfu Turbo DNA polymerase	Invitrogen
<i>PfuTurbo</i> [®] DNA Polymerase	Stratagene [®]
PK	Sigma
Platinum [®] <i>Taq</i> DNA Polymerase HiFi	Invitrogen
Sal I	New England Biolabs
Spe I	New England Biolabs
Sph I	New England Biolabs
T4 DNA ligase	Invitrogen
T4 DNA ligase	New England Biolabs
Taq DNA polymerase	Invitrogen
Thrombin	Novagen
Trypsin	Roche
Xba I	New England Biolabs

Overview of Microorganisms

Competent Cells	Source
BL21-Gold cells	Stratagene®
DH5- α	Laboratory Strain (Invitrogen)
DH10Bac™	Invitrogen
AX3-Orf ⁺	Laboratory Strain

Overview of Columns and Resins for Protein Purification

Columns	Source
MonoS™ (FPLC)	Pharmacia Biotech, PA25031
MonoQ™ (FPLC)	Pharmacia Biotech, ID 0139190
Superdex 200 (FPLC)	Pharmacia Biotech, (HR 10/30)
Ni-NTA Superflow Resin (FPLC)	Qiagen (30430)
Anti FLAG® M2 Affinity Resin (Batch)	Sigma-Aldrich, A2220

Overview of the Kits

Kits	Source
QIAprep® Spin Miniprep Kit	Qiagen
QIAquick® Gel Extraction Kit	Qiagen
QuikChange® Site-directed Mutagenesis Kit	Stratagene®
Bradford protein assay	Bio-Rad Laboratories

Molecular Structure of the Dyes

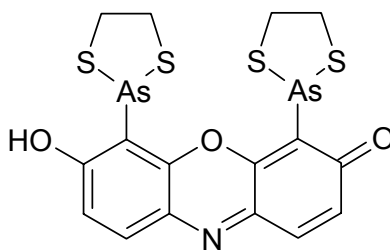


Figure 69: Molecular structure of the ReAsH-EDT₂ dye. In a protein- ReAsH-EDT₂ dye reaction the sulfur atoms on the cysteine residues (CCRECC) replace the sulfur atoms in the ReAsH-EDT₂ dye.

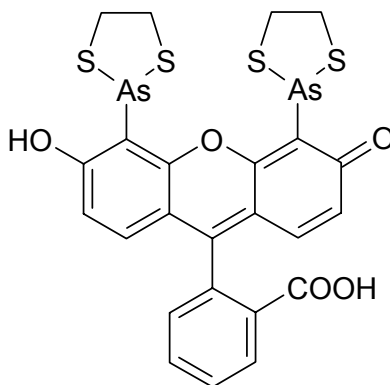


Figure 70: Molecular structure of the FlAsH-EDT₂ dye. In a protein- ReAsH-EDT₂ dye reaction the sulfur atoms on the cysteine residues (CCRECC) replace the sulfur atoms in the ReAsH-EDT₂ dye.

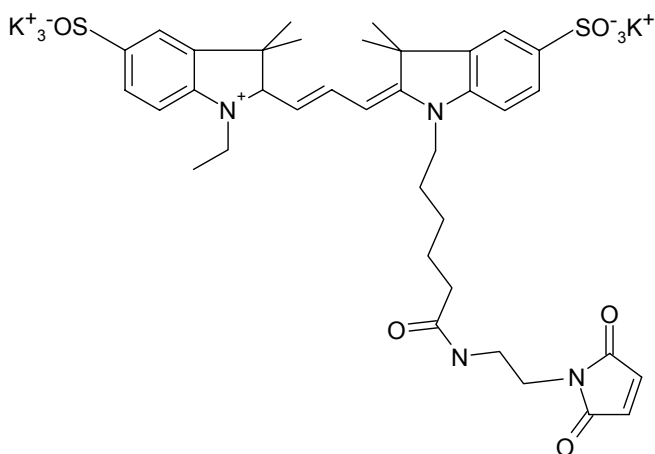


Figure 71: Molecular structure of the Cy3 dye.

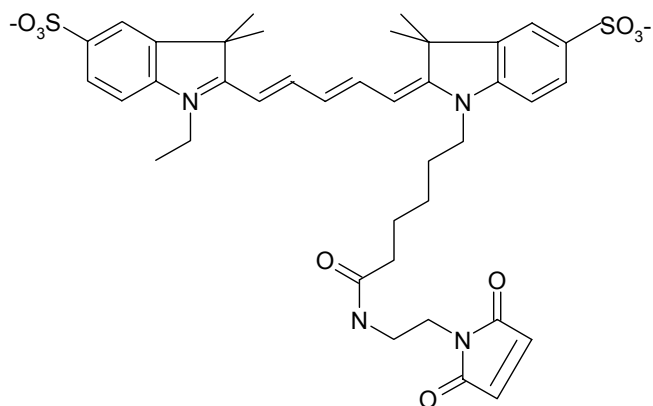


Figure 72: Molecular structure of the Cy5™ dye.

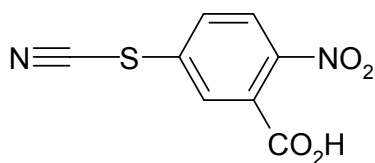


Figure 73: Molecular structure of the NTCB.

Abbreviations

ADP	Adenosine 5'-diphosphate
ADP*VO ₄	ADP vanadate
ADPBeF _x	ADP-beryllium fluoride
AESF	4-(2-Aminoethyl)benzenesulfonyl fluoride hydrochloride
AMP*PNP	Adenyl-5'-yl imidodiphosphate
APS	Ammonium peroxodisulfate
ATP	Adenosine 5'-triphosphate
Bluo-gal	halogenated indolyl-β-galactoside
Bp	Base pair
BSA	Bovine serum albumin

ddH ₂ O	Double distilled water
DMPS	2,3-Dimercapto-1-propanesulfonic acid sodium salt monohydrate
DNA	Deoxyribonucleic Acid
DTT	Dithiothreitol
EDT	1,2 ethanedithiol
EDTA	Ethylenediaminetetraacetic acid
EGTA	Ethylene-bis(oxyethylenenitrilo) tetraacetic acid
ELC	Essential light chain
EMCCD	Electron multiplying charge coupled detector
FBS	Fetal bovine serum
FIAsH	4,5-Bis(1,3,2-dithiarsolan-2-yl)-fluorescein
FPLC	Fast performance liquid chromatography
GuHCl	Guanidium hydrochloride
HC	Heavy chain
HEPES	4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid
HMM	Heavy meromyosin
HPLC	High pressure liquid Chromatography
IPTG	Isopropyl-b-D-thiogalactopyranoside
LB	Luria broth
LMM	Light meromyosin
MES	2-Morpholinoethanesulfonic acid
MWCO	Molecular weight cut off
NEB	New England Biolabs
NEM	N-Ethylmaleimide
NIH	National institute of health

NTCB	2-Nitro-5-thiocyanatobenzoic acid
PCR	Polymerase chain reaction
PMSF	Phenylmethanesulfonyl fluoride
pN	Pico Newton
ReAsH	4,5-Bis(1,3,2-dithiarsolan-2-yl)-resorufin
RLC	Regulatory light chain
Rpm	Revolution per minute
RT	Room temperature
S.O.C	Super Optimal Catabolite
SDS	Sodium dodecyl sulfate
TAE	Tris-Acetate-EDTA
TCA	Trichloroacetic acid
TCEP	Tris-(2-Carboxethyl) phosphine
TEMED	N,N,N',N'-Tetramethylethylenediamine
TFA	Trifluoroacetic acid
TIRF	Total internal reflection fluorescence
TLCK	1-Chloro-3-tosylamido-7-amino-L-2-heptanone hydrochloride
TMR	Tetramethylrhodamine
TPCK	(S)-1-Chloro-4-phenyl-3-tosylamido-2-butanone
TRITC	tetramethylrhodamine isothiocyanate
Wt	Wild type
YFP	Yellow fluorescent protein
β ME	β -Mercaptoethanol

Amino Acid Sequences of the Protein Constructs

Porcine Myosin VI Heavy Chain

MEDGKPVWAPHPTDGFQVGNIVDIGPDSLTIIEPLNQGKTFALINQVFPAAEEDSKKDVEDNCSLM
YLNEATLLHNIKVRYSKDRIYTYVANILIAVNPYFDIPKIYSSETIKSYQGKSLGTMPPHVFAIADKAFRDM
KVLKLSQSIIVSGESGAGKTENTKFVLRYLTESYGTGQDIDDRIVEANPLLEAFGNAKTVRNNNSSRF GK
FVEIHFNEKSSVVGGFVSHYLLEKSRICVQGKEERNYHIFYRLCAGASEDIRERLHLSPPDNFRYLN RGC
TRYFANKETDKQILQNRKSPEYLKAGSLKDPLLDHGD FIRMCTAMKKIGLDDEEKLDLFRVVAGVLH
LGNIDFEEAGSTSGGCNLKNKSTQALEYCAELLGLDQDDL RVSLTRVMLTTAGGAKGTVIKVPLKVE
QANNARDALAKTVYSHLFDHVVNRVNQCFFETSSYFIGVLDIAGFEYFEHNSFEQFCINYCNEKLQ
QFFNERILKEEQELYQKEGLGVNEVHYVDNQDCIDLIEARLVGILDILDEENRLPQPSDQHFTSAVHQ
KHKDHFRLSIPRKSCLAHRNIRDDEGFIRHFAGAVCYETTQFVEKNNDALHMSLES LICESRDKFIRELFE
SSTNNNKDTKQKAGKLSFISVGNKFKTQLNLLLDKLRSTGASFIRCIKPNLKMTSHHFEGAQILSQLQCS
GMVSVLDLMQGGFSPRASFHLYNMYKKYMPDKLARLDPRLFCALFKALGLNEIDYKFG LTKVFFR
PGKFAEFDQIMKSDPDHLAELVKRVNHWLICSRWKKVQWC SLSVIKLNKIKYRAEACIKMQKTIRM
WLCKRRHKPRIDGLVKVGTLLKRLDKFNEVVSALKD GKQEMSKQVRDLEISIDALMAKIKSTMMTRE
QIQKEYDALVKSSAVLLSALQKKKQEEEEAERLRRIQEEMEKERKRREEDEQRRRKEEEERRMKLEME
AKRKQEEEEERKRDDDEKRIQAEVEAQLAR
MKQLEDKVEELLSKNYHLENEVARLKKLVGEVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGD
ATYGKLTLLKFICTGKLPVPWPTLVTTFGYGVQC FARYPDHMRQHDFFKSAMPEGYVQERTIFFKDD
GNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYN SHNVYIMADKQKNGIKVNFKIRHNIE
DGSVQLADHYQQNTPIGDGPVLLPDNHYSYQSALS
KDPNEKRDHMLLEFVTAAGITLGMDELYKGGDYKDDDDK

Color coding: GCN4, YFP, FLAG tag

Chicken Myosin V Heavy Chain

MDYKDDDDKGAASELYTKYARVWIPDPEEVWKS AELLKDYKPGDKVLQRLLEEGKDLEYCLDPKTK E
LPPLRNPDILVGENDLTALSYLHEPAVLHNLKVR FIDSKLIITYCGIVLVAINPYEQLPYGEDIIINAYS GQ
NMGDMDPHIFAVAEAYKQMARDERNQSIIVSGESGAGKT VSAKYAMRYFATVSGSASEANVEEK
VLASNPIMESIGNAKTRNDNSSRF GKYEIGFDKRYRIIGANMRTYLLEKSRVVFQAE EERNYHIFYQLC
ASAALPEFKTLRLGNANYFH YTKQGGSPVIDGIDDAKEMVNTRQACTLLGISDSYQMGIFRILAGILH
LGNVEFASRSDSCAIPPKHDPLTIFCDLMGVDYEEMAHWLCHRKLATATETYIKPISKLHAINARDAL
AKHIYANLFNWVDHVNKALHSTVKQHSFIGVLDIYGFETFEINSFEQFCINYANEKLQQQFNMHVFKL
EQEYMKEQIPWTLIDFYDNQPCINLIEAKMGVLDLLDEECKMPKGSDDTWAQKLYNTHLNKCALFE

KPRLSNKAFIIKHFADKVEYQCEGFLEKNKDTVYEEQIKVLKSSKKFKLLPELFQDEEKAISPTSATPSGRV
PLSRTPVKPAKARPGQTSKEHKKTVGHQFRNSLHLLMETLNATTPHYVRCIKPNDKFPFTFDEKRAVQ
QLRACGVLETIRISAAGFPSRWTYQEFFSRYVLMKQKQDVLSDRKQTCKNVLEKLILDKDKYQFGKTKI
FFRAGQVAYLEKIRADKLRAACIRIQKTIRGWLMRKKYMRMRRAAITIQRVVRGHQARCYATFLRRT
AIIIIQKFQRMVYVRKRYQCMRDATIALQALLRGYLVRNKYQMMLREHKSIIQKHVRGWLARVHYH
RTLKAIYYLQCCYRRMMAKRELKLLKIEARVERYKLLHIGLENKIMQLQRKVDEQNKDYKCLMEKLT
NLEGVYNSETEKLRNDVERLQLSEEEAKVATGRVLSLQEEIAKLRKDLEQTRSEKKSIEERADKYKQETD
QLVSNLKEENTLLKQEKETLNHRIVEQAKEMTETMERKLVETKQLELDLNDERLRYQNLNNEFSRLEERY
DDLKEEMTLMLNVMKQLEDKVEELLSKNYHLENEVARLKKLVGEVSKGEELFTGVVPILVELDGDVN
GHKFSVSGEGEGDATYGKLTLCFICTTGKLPVPWPTLVTTFGYGVQCFARYPDHMRQHDFFSAMP
EGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQ
KNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYSYQSALS KDPNEKRDHMLVLEFV
TAAGITLGMDELYK

Color coding: GCN4, YFP, FLAG tag

Dictyostelium discoideum Myosin II S1 Heavy Chain

MNPIHDRSDYHKYLKVKQGSDLFKLTVSDKRYIWYNPDPKERDSYECGEIVSETSDFSFTFKTVDGQD
RQVKDDANQRNPIKFDGVEDMSELSYLNPAVFHNLVRVYNQDLIYTYSGLFLVAVNPFKRIPIYTQE
MVDIFKGRRRNEVAPHIFAISDVAYRSMDDRQNSLLITGESGAGKTENTKKVIQYLASVAGRNQA
NGSGVLEQQILQANPILEAFGNAKTRNNNSSRFGKFIEIQFNNAGFISGASIQSYLLEKSRVVFQSETE
RNYHIFYQLLAGATAEEKALHLAGPESFNLYNQSGYVDIKGVSDSEEFKTRQAMDIVGFSQEEQMS
IFKIIAGILHLGNIKFEKGAGEGAVLKDKTALNAASTVFGVNPVLEKALMEPRILAGRDLVAQHLNVE
KSSSRDALVKALYGRFLWLVKKINNVLCQERKAYFIGVLDISGFEIFKVNSEFQLCINYTNEKLQQFF
NHHMFKLEQEEYLKEKINWTFIDFGLDSQATIDLIDGRQPPGILALLDEQSVFPNATDNTLITKLHSHFSK
KNAKYEPRFSKTEFGVTHYAGQVMYEIQDWLEKNKDPLQQDLELCFKDSSDNVVTKLFNDPNIASR
AKKGANFITVAAQYKEQLASLMATLETTNPHFVRCIIPNNKQLPAKLEDKVVLDQLRCNGVLEGIRITR
KGFPNRIIYADFKRYLLAPNVPRDAEDSQKATDAVLKHLNIDPEQYRFGITKIFFRAGQLARIEEARE
QRISEIKAIQAATRGWIARKVYKQAREHTVAARIQQNLRAYIDFKSWPWWKLFKARPLLKRRNFEKE
IKEKEREILEKSNLTDSTTQDKLEK

Dictyostelium discoideum Essential Light Chain

MSASADQIQECFSIFDKDNDGKVSVEDIGACLRLGKSPTMADIEALKTEIGAKEFDINTLKSIIKPNIK
TPQEQQKEMLDFAKALDKEGHGTIQGAELRQLLTLGDYLSAEVDELFEISVDSTTGAVSYASLVNTI
VSGYPEFRHKFQSGFRVKR EHYHQF

***Dictyostelium discoideum* Regulatory Light Chain**

MASTKRRLNREESSVVLGEEQVAELKEAFELFDKDRGTGFIKKDALKTTCKGFGVFMEDQLDAMFAE
ADTKSGAIGFPEFMSMMSRRMKQTSNEQILMNAFKTDPEGNGYILTKDISKALTTLGDKLTEAELGELL
SISENEQKQVKYDLFVNLFSSK

***Drosophila melanogaster* Calmodulin**

MADQLTEEQIAEFKEAFSLFDKDGDTITTKELGTVMRSLGQNPTEAELQDMINEV
DADGNGTIDFPEFLTMMARKMKDTSSEEEIREAFRVFDKDGNGYISAAELRHVMTNL
GEKLTDEEVDEMIREADIDGDGQVNYEEFVQMMTAK