

2. Materials and Solutions

Abbreviations

Chemicals/ Material		Terms	
2D	2-Dimensional-	Acc. Nr.	Accession-Nummer
amc	Aminomethylcumarin	Apaf-1	<i>Apoptotic Protease Activating Factor-1</i>
aomk	Arylmethylketon	BAD	<i>Bcl-Associated-Protein</i>
APS	Ammonium persulfate	Bcl	<i>B-Cell Lymphoma Gene2 Product</i>
ATP	Adenosyl-Triphosphate	BH	Bcl-2-Homologie-Domain
bio-	biotinyl-	CAD	<i>Caspase-Activated-DNase1</i>
BPB	Bromphenol blue	cAMP	cycloAdenosylmonophosphat
BSA	Bovine-Serum albumin	CARD	<i>Caspase Recruitment Domain</i>
cAMP	cyclic-Adenosylmonophosphate	Casp.	Caspase/ n
CAPS	Cyclohexylaminopropansulfonacid	CE	Cytosolic Extract
CHAPS	3-[(3-Cholamidopropyl)- dimethylamino]-1-propansulfonat	CrmA	<i>Cytokine-Response-Modifier A</i>
cho	Aldehyd	DED	<i>Death Effector Domain</i>
cmk	Chlormethylketon	DNA	Desoxyribonukleinsäure
dNTP	deoxyribonucleoside triphosphate	IAP	<i>Inhibitors of Apoptosis</i>
DEVD	Aspartyl-glutamyl-valyl-aspartate	ICAD	<i>Inhibitor of CAD</i>
DMSO	Dimethylsulfoxid	mcs	multiple cloning site
DTT	Dithiothreitol	MAP-	<i>Mitogen-Activated-Protein-</i>
<i>E.coli</i>	<i>Escherichia coli</i>	PCR	<i>Polymerase-Chain-Reaction</i>
EDTA	Ethylendiamintetraacetate	TNF	Tumor-Necrosis-Factor
GFP	<i>Green-Fluorescence-Protein</i>	UV	Ultraviolett
EGTA	Bis-(aminoethyl)-glykolether- N.N.N',N'-tetraacetacid	$\Delta\Psi$	Transmembranpotential
FACS	Fluorescence Activated Cell Sorter	h	human
FCS	Fetal-Calf Serum	m	mouse
GFP	<i>Green-Fluorescence- Protein</i>	mt	mutant
fmk	Fluormethylketon	wt	wild type
HEPES	N-2 Hydroxyethylpiperazin-2' ethansulfonacid		
IETD	Isoleucyl-glutamyl-threonyl- aspartate		
IPTG	Isopropyl- β -D-thiogalactosid		
Luminol	3-Aminophtalhydrazid		
LB	Luria Broth		
PBS	Phosphate Buffered Saline	Units	
PMSF	Phenylsulfonylchlorid	μ M	micro molar
pNa	para-Nitroanilin	μ g	micro gram
		mM	mili molar
		bp	Base pair
		kDa	kiloDalton
		MW	Molecular weight
		pmol	pico Mol
		U	Unit
		V	Volt
		W	Watt
RNAasin	RNase-Inhibitor		
SDS	Na-Dodecylsulphate		
SSC	Natrium-Chlorid/-Citrat		
TBE	Tris/Boric acid/EDTA		
TCA	Trichloroaceticacid		
TEMED	N,N,N',N'- Tetramethylethylendiamin		
VEID	Valyl-glutamyl-isoleucylaspartate		
YVAD	Tyrosyl-vanyl-alanyl-asparstate		

2.1 Enzyme and Kits

2.1.1 Restrictionendonucleases

Tab 2.1. All Restrictions endunucleases which were used during the work.

BamH I	Buffer BamHI	MBI Fermentas, St. Leon Roth
EcoRI	Buffer EcoRI	MBI Fermentas, St. Leon Roth
Eco 3I-1	BufferY ⁺ Tango	MBI Fermentas, St. Leon Roth,
Esp 3I-1	BufferY ⁺ Tango	MBI Fermentas, St. Leon Roth
Dpn I	NEBuffer 4	New England Biolabs
Xho I	Buffer R ⁺	MBI Fermentas, St. Leon Roth

Buffer EcoRI : 50mM Tris-Cl (pH 7.5)
 10mM MgCl₂
 100mM NaCl
 002% Triton 100
 +0.1mg/ml BSA, at 37°C incubation

Buffer BamHI : 10mM Tris-Cl (pH 8.0)
 5mM MgCl₂
 100mM KCl
 0.02% Triton 100
 +0.1mg/ml BSA, at 37°C incubation

Buffer Y⁺ Tango : 33mM Tris-Acetat (pH 7.9)
 10mM Mg-Acetat
 66mM K-Acetat
 +0.1mg/ml BSA, at 37°C incubation

Buffer R⁺ : 10mM Tris-Cl (pH 8.0)
 5mM MgCl₂
 100mM KCl
 0.02% Triton 100
 +0.1mg/ml BSA, at 37°C incubation

NEBuffer 4 : 20 mM Tris-Acetat (pH 7.9)
 10mM Mg-Acetat
 50mM K-Acetat
 1mM DTTat 37°C incubation

2.1.2 DNA-Modifying enzymes and kits

Tab 2.2. Enzymes and Kits, for Modification of Nucleicacids

Pfu Turbo® Taq Polymerase	STRATEGENE
Hot Star Taq-Polymerase	QIAGEN
Alkaline Phosphatase (CIP)	MBI Fermentas, St. Leon Roth

2.1.3 Other enzymes

Tab 2.3. Other Enzymes

Benzo-Nuclease	Merck, Darmstadt
RNAasin TM	Promega Boehringer Ingelheim
RNase A	Macherey & Nagel, Düren

2.2 Plasmids

pET21b :

pET-21b(+) is a 5442bp plasmid; carries an N-terminal T7•Tag® sequence plus an optional C-terminal His•Tag® sequence. Selectable marker is ampicillin resistance. Unique sites are shown on the circle map. The sequence is numbered by the pBR322 convention, so the T7 expression region is reversed on the circular map. The cloning/expression region of the coding strand transcribed by T7 RNA polymerase is shown. The f1 origin is oriented so that infection with helper phage will produce virions containing single-stranded DNA that corresponds to the coding strand. Therefore, single-stranded sequencing should be performed using the T7 terminator primer .

Accession number: 69337-3 Novagen.

pQBI25- fN3:

The pQBI25- fN3 is a 6243 bp plasmid which is designed for the generation of fusions with the amino terminus of Quantum' s AFPs™. It contains a comprehensive MCS in one of the three reading frames, separated from the AFPs™ with a flexible peptide linker. The absence of initiation codon facilitates the expression of native protein sequences containing endogenous ATG initiation codons without any risk of transcription initiation at downstream sites. The vector has been designed as functional cassettes, allowing the isolation of the promoter region, AFP cds, polyA signal sequence or any combination thereof. The pQBI25 vector contains Quantum' s SuperGlo™ GFP (Green Fluorescent Protein).

Accession number: AFP2213 Quantum Biotechnologies

2.2.1 Plasmid-constructs

pcDNA+Casp-3: The caspase-3 containing pcDNA construct was used as template to amplify mouse caspase-3 insert for cloning into the expression vector. The construct has previously prepared by Dr. Viviane Hoppe.

2.1 Oligonucleotides

During this work the following oligonucleotides were used. The synthesis of the oligos were ordered to the company Sigma ARK (Darmstadt).

Tab 2. 4. List of Oligonucleotides : Melting temperature (T_D) was calculated form the following equation

NAME	SEQUENCE 5' -> 3'	T_m	Specificity	↔	Acc.
m Casp3 2-1	CggTCTCggATCggAgAACAAACAAAACCTCA	90	Dove tail, BamH1 (Eco311)	→	
m Casp3 2-2	CggTCTCCTCgAgATAAAAgtACAgTTCTTTCg T	90	Dove tail, Xho1 (Eco311)	←	
m Casp3- EcoRIr	CggTCTCgAATTCgTgATAAAAgtACAgTTCTT T	94	Dove tail, EcoRI	←	
Casp3-4-1-P	CggTCTCggATCACCATggagAACAAACAAAACC TCA	108	Dove tail, BamHI and ATG	→	
m Casp3-mt C-A f	ATCATTcag gCCgCCCggggTACggAG	90	Mutagenesis, C/A 163	→	
m Casp3-mt C-A r	CTCCgTACCCCGggCggCCTgAATgAT	90	Mutagenesis, C/A 163	←	
m Casp-12 C-A	CATgCAggCCgCCAgAggCAgATA	78	Mutagenesis, C/A 925	→	
m Casp-12 C-A rev	TATCTgCCTCTggCggCCTgCATg	78	Mutagenesis, C/A 925	←	
m Casp-12 mit	TCCATTTACAgCTCAACACAC	60	Middle of Casp12 1001	←	
v T7 ter-rev	gCTAgTTATTgCTCAgCgg	58	T7 terminator site	←	
v T7 ter- revII	AAGACCCgTTTAgAggCCC	60	T7 terminator site	←	
v His-Topo T7	TAATACgACTCACTATAggg	56	Sequencing, euk. Topo vector	→	
v His-Topo BGH-re	TAATACgACTCACTATAggg	54	Sequencing, euk. Topo Vector	←	
v PIRES-2- SEQ	CAAATgggCggTAggCgtg	62	Sequencing, PIRES-2, pEGFP N1, pDsred N1	→	
v PIRES-2- REV	TATAgACAAACgCACACCgg	60	Sequencing, PIRES-2	←	

$T_D = [(C_n + G_n) \times 4 + (A_n + T_n) \times 2]^\circ\text{C}$, (n = number of basepairs)

→: Primer in 5'-Direction, ← Primer in 3'-Direction; v=vector, m=murin; Acc.= Accession Nr.

2.2 Antibodies

Tab 2.5. Antibodies for Western Blottings.

1. Antibody	Produkt Nr.	Verdünnung	Anbieter
α - Caspase2, Rabbit IgG	06-537-MN	1 μ g/ml	Upstate/ Biomol, Hamburg
α - Caspase2, Maus IgG	I29120	1:5000	Dianova, Hamburg
α - Caspase3, Rabbit IgG	06-735-MN	1 μ g/ml	Upstate/ Biomol, Hamburg
α -Caspase3p17 Rabbit IgG	9661S	1 μ g/ml	New England Biolabs
α - Caspase6, Rabbit IgG	218774	1:750	Calbiochem,
α - Caspase9, Rabbit IgG	(H-83) sc-7885	1:250	Santa Cruz/ Biomol, Hamburg
α -Caspase 12 Rat IgG	-	1:50	Dr. Yuan/
α -Cytochrom C, Maus IgG	65981A	1 μ g/ml	Pharmingen
Apaf-1, Rabbit IgG	611364	1 μ g/ml	BD Transduction
Grp 78, Rabbit IgG-AP	SPA-826	1:500	StressGen
Anti-20S Proteasome, α -Subunit	B31545	1:20 000	Calbiochem
Anti-His Tag Antibody	70796-4	0.2 μ g/ml	Novagen
2.Antibody			
Peroxidase-conjugated Sheep-Anti-Maus IgG	515-035-071	1:10000	Dianova, Hamburg
Peroxidase-conjugated Goat-Anti-Rabbit Ig	111-035-003	1:5000	Dianova, Hamburg
Peroxidase-conjugated Rabbit-Anti-Rat Ig	44624	1: 10000	Dianova, Hamburg
Cy TM 3-conjugated Affine pure F(ab) ₂ Goat Anti-Rabbit	111-166-008	1 μ g/ml	Dianova, HAMBURG
Affinitylabeling			
Peroxidase-conjugated-ExtrAvidin	E-2886	1:2000	Sigma Immuno Chemicals

2.3 Inhibitors and Substrates for Caspases

Tab 2.6. Inhibitores for Caspases

Inhibitores	
Ac-YVAD.cmk	Bachem, Heidelberg
Z-DEVD.cmk	Bachem, Heidelberg
Z-VEID.fmk	Enzyme Systems, Livermore (CA)
Ac-YVAD.cho	Bachem, Heidelberg
Ac-DEVD.cho	Bachem, Heidelberg
Ac-VEID.cho	Bachem, Heidelberg
Ac-IETD.cho	Bachem, Heidelberg
Ac-YVK(bio)D.aomk	Bachem, Heidelberg

Tab 2.7. Substrates for Caspases

Z-DEVD.pNa	Bachem, Heidelberg
Z-VEID.pNa	Bachem, Heidelberg
Z-IETD.pNa	Bachem, Heidelberg

2.4 Molecular weight markers

2.5.1 Molecular weightmarkers for proteins

These markers were used for SDS-PAGE. The individual proteins were prestained, and they were visible on gel or on the membrane after blotting.

SDS 7 Molecular weight marker from Sigma: (in kDa)

66- 45 - 36 - 29 - 24 - 20 - 14

prestained Benchmark BRL from GIBCO: (in kDa)

178 – 114 – 82 - 62* - 49 – 37 – 25 – 19 – 14 – 9

2.6.2 Molecular weight marker for DNA

LMS (100bp-DNA Ladder): This marker was supplied from MBI-Fermentas. Size of the fragments are follows as basepairs(bp):

1000 - 900 - 800 - 700 - 600 - 500 - 400 - 300 - 200 - 100 - 80

MMS: This marker was supplied from MBI-Fermentas. The marker was prepared by digestion of λ -DNA with the enzym *Eco130I* (*StyI*). Size of the fragments are follows as basepairs(bp):

19329 - 7743 - 6223 - 4254 - 3472 - 2690 - 1882 - 1489 - 925 - 421 - 74

2.5 Sequence informations

Tab. 2.8. Accessions-Numbers for Sequence informations from NCBI-Databank

Caspases	NCBI-Accession-Nummer	
	human	murin
Casp-1	U13697-U13700	L03799
Casp-2	D28492	Y13085
Casp-3	U13737-U13738	Y13086
Casp-6	U20537	Y13087

Tab. 2.8. Accessions-Numbers continued

Casp-7	U39613	Y13088
Casp-8	U60520	AF067834
Casp-9	U60521	ABO19600
Casp-10	U60519	--
Casp-11	--	Y13089
Casp-12	--	Y13090
Casp-13	NM_003723	--
Casp-14	NM_012114	AJ007750

2.6 Chemicals

3-Mercaptopropionic acid	Serva, Heidelberg
5-Brom-4-chlor-3-indolyl- β -D-galaktopyransid (X-Gal)	Alligene, Illkirch (F)
8Br-cAMP	Sigma, Deisenhofen
Acrylamid	Serva, Heidelberg
Adenosin	Sigma, Deisenhofen
Agarose for DNA-Elektrophoresis	Serva, Heidelberg
Aminomethylcoumarin (amc)	Sigma, Deisenhofen
Ammonumperoxodisulfat (APS)	Sigma, Deisenhofen
Ampholyte, pH 3-10	Serva, Heidelberg
Ampicillin	Serva, Heidelberg
Ampuwa-H ₂ O	Fresenius, Bad Homburg
Anisomycin	Sigma, Deisenhofen
A23187 (Ionophore)	Calbiochem
Bacto-Agar	Gibco/BRL, Eggenstein
Bicinchoninicacid (BCA)	Sigma, Deisenhofen
Borat	Roth, Karlsruhe
Bromcyan	Sigma, Deisenhofen
Bromphenolblau	Serva, Heidelberg
Bug Buster Reagent	Novagen
BSA	Serva, Heidelberg
CAPS-Buffer	Roth, Karlsruhe
CHAPS	Calbiochem,
Chloroform	Ferak, Berlin
Colchicin	Sigma, Deisenhofen
Coomassie G	Serva, Heidelberg
Cover Fluid	Pharmacia, Freiburg
DEPC	Sigma, Deisenhofen
Desoxyribonukleosid-5'-triphosphat-Set (dATP, dCTP, dGTP, dTTP)	Boehringer, Mannheim
Dimethyldichlorsilan	Merck, Darmstadt
Dimethylformamid	Fisons, Loughborough (UK)
DiSodiumcarbonat	Sigma, Deisenhofen

DiSodiumhydrogenphosphatdihydrat	Merck, Darmstadt
DMSO	Sigma, Deisenhofen
DTT	Sigma, Deisenhofen
Dynabeads, polydT	Dynal
EDTA (Titrplex III)	Merck, Darmstadt
EGTA (Titrplex VI)	Merck, Darmstadt
Aceticacid 99%	Merck, Darmstadt
Ethanol p.a.	Ferak, Berlin
Ethidiumbromide	Roth, Karlsruhe
Ficoll	Sigma, Deisenhofen
Formaldehyd for Silver stain 37%	Fluka, Buchs
Formaldehyd min. 37%	Merck, Darmstadt
Formamid, deionised	Gibco/BRL, Eggenstein
Forskolin	Sigma, Deisenhofen
Glutamin	Sigma, Deisenhofen
Glycerin	Roth, Karlsruhe
Glycin	Merck, Darmstadt
Urea	Sigma, Deisenhofen
Hefe-Extrakt	Gibco/BRL, Eggenstein
HEPES	Roth, Karlsruhe
Heringssperma-DNA	Boehringer, Mannheim
Hyclone Calfserum	Hyclone, Logan (USA)
Immidazole	Sigma-Aldrich
Immobiline Strip	Pharmacia, Freiburg
Inhibitormix, EDTAfree	Boehringer, Mannheim
Iodacetamid	Sigma, Deisenhofen
IPTG	Sigma, Deisenhofen
Isoamyl alcohol	Merck, Darmstadt
Isopropanol	Ferak, Berlin
Potassiumacetat	Roth, Karlsruhe
Potassiumchlorid	Roth, Karlsruhe
Potassiumdihydrogenphosphat	Ferak, Berlin
Potassium-Sodium-Tartrat x4 H ₂ O	Merck, Darmstadt
Kanamycin	Sigma, Deisenhofen
Coppersulfat x 5 H ₂ O	Sigma, Deisenhofen
Leupeptin	Sigma, Deisenhofen
Lithiumchlorid	Sigma, Deisenhofen
Luminol (3-Aminophtalhydrazide)	Fluka, Neu-Ulm
Magnesiumchlorid	Sigma, Deisenhofen
Mc Coy 5A, Powder media	Gibco/BRL, Eggenstein
MCDB 402, Powder media	Gibco/BRL, Eggenstein
Mercaptoethanol	Serva, Heidelberg
Methanol	Roth, Karlsruhe
MOPS-Buffer	Roth, Karlsruhe
N,N`-Methylenbisacrylamid	Serva, Heidelberg
Sodiumacetat-trihydrat	Merck, Darmstadt
Sodiumazid	Serva, Heidelberg
Sodiumchlorid	Roth, Karlsruhe
Sodiumdihydrogenphosphat-monohydrat	Merck, Darmstadt
Sodiumhydrogencarbonate	Roth, Karlsruhe

Sodiumhydroxid	Merck, Darmstadt
Sodiumlaurylsarcosin	Sigma, Deisenhofen
Nonidet P40LKB	Sigma, Deisenhofen
Okadaacid	Calbiochem
OptiMem	Gibco/BRL, Eggenstein
Paraffinoil for PCR	Roth, Karlsruhe
p-Cumaracid	Sigma, Deisenhofen
Perchloracid	Merck, Darmstadt
Phenol, pH 7,5-8,0	Roth, Karlsruhe
Phenol, watersaturated, pH 5,5	Appligene, Illkirch (F)
Phenolred	Sigma, Deisenhofen
PMSF	Sigma, Deisenhofen
p-Nitroanilin	Sigma, Deisenhofen
Polyvinylpyrrolidon	Sigma, Deisenhofen
Protein-A-Sepharose	Pharmacia, Heidelberg
Protein-Marker SDS 7B	Sigma, Deisenhofen
Rotiphorese	Roth, Karlsruhe
RPMI	Sigma, Deisenhofen
Saccharose	Serva, Heidelberg
Salzacid, 37%	Ferak, Berlin
SDS	Serva, Heidelberg
Sephadex G50	Pharmacia, Heidelberg
Silverniträt	Sigma, Deisenhofen
Source Q	Pharmacia, Heidelberg
Talon Cobalt Metal resin	Clontech
TEMED	Serva, Heidelberg
Thapsigargin	Calbiochem
Thiourea	Sigma, Deisenhofen
Trichloraceticacid	Roth, Karlsruhe
Tricin	Roth, Karlsruhe
TriSodiumcitrat-dihydrät	Merck, Darmstadt
Tris-Buffer	Roth, Karlsruhe
Triton-X100	Serva, Heidelberg
Trypsin-EDTA-Solution	Gibco/BRL, Eggenstein
Trypton/Pepton	Gibco/BRL, Eggenstein
Waterstoffperoxid 35% (v/v)	Roth, Karlsruhe
Xylencyanol	Sigma, Deisenhofen

2.7 Equipments

Analog-Digitalwandler	Perkin-Elmer, Langen
Incubator for Cell Culture	Heraeus/Sepatech, Hanau
Crocodil II for PCR	Appligene, Illkirch (F)
Electrophoresis apparatus for 2D Gelelectrophoresis	Institutsworkshop
Electrophoresiscomb for horizontal Agarosegels	Institutsworkshop
Electrophoresiscombs for Minigels	Bio-Rad, München
ELISA-Reader	

FACS, Epics Elite ESP	Coulter
Fluorescence-Microscope Leica <i>DM IRB</i>	Leica
Fluorspektrophotometer Flouromax SPEX	SPEX
Fractioncollector 203	Gilson/ABIMED, Langenfeld
HPLC-Anlage: L6210-Intelligent Pump	Merck/Hitachi, Darmstadt
L4000 UV-Detektor	Merck/Hitachi, Darmstadt
LKB Ultrascan XL	Pharmacia, Heidelberg
Pegasus Semi-Dry Blot	Phase, Lübeck
Polytron PT100 Ultrathorax	Kinematica, Littau
Rockomat (Shaker)	Tecnomara, Zürich (CH)
Rontgenfilm developer	Konica, Tokio (J)
Schleifeninjektor	LATEK, Heidelberg
Printer	LATEK, Heidelberg
Power supply E752	Consort, Turnhout (B)
Power supply Phero-stab 500	Biotec-Fischer, Reiskirchen
Speed Vac	Savant (Bachhofer, Reutlingen)
Sterilbank "Gelaies"	Flow Laboratories, Meckenheim
Sephacryl S 300 Coloumn	Pharmacia
Superose 6 HR Coloumn	Pharmacia
Superloop	Pharmacia, Heidelberg
Thermal-Cycler Gene-Amp 2400 for PCR	Perkin-Elmer, Langen
Benchcentrifuge	Heraeus/Sepatech, Hanau;
Ultracentrifuge L8-70M	Beckman, München
UV-Handlamp	Vilbert Lourmat, Marne La Vallee (F)
UV-Spektralphotometer Uvikon 930	Kontron, Eching
UV-Stratalink 1800	Stratagene, Heidelberg
Centrifuge Megafuge	Heraeus/Sepatech, Hanau

2.8 Other Materials and Membranes

Konika X-Ray Film, "medical"	Konika, Tokio (J)
Nitrocellulose BAS 85	Schleicher & Schuell, Dassel
Nylonmembrane Hybond-N	Amersham, Braunschweig
PCR Purification Kit	Quiagen, Hilden;
PCR-Reactionstubes (0,2ml)	Macherey & Nagel, Düren
	Perkin-Elmer, Langen;
	Roth, Karlsruhe
Pipette tips	Greiner, Frickenhausen
Plasmid Purification Kit	Quiagen, Hilden;
	Macherey & Nagel, Düren
PVDF-Membrane	Applied Biosyst. Darmstadt
Reactionstubes (0,5ml)	Hartenstein, Würzburg
Reactionstubes (1,5ml and 2ml)	Eppendorf, Hamburg;
Sterilfilter	Roth, Karlsruhe
Whatman-Paper	Schleicher & Schuell, Dassel
Cell culture flasks, -vessels and -plates	Greiner, Frickenhausen
Centrifuge Falcons (15ml and 50ml)	Greiner, Frickenhausen