

## PRÍLOHA : POUŤITÉ CHEMIKÁLIE, KITY, PRIMERY

Biochemické metódy:

### Fluorogenetické substráty a inhibítory peptidáz

Z-Phe-Arg-AMC	Bachem
Z-Arg- Arg- AMC	Bachem
CA-074	L-3-trans-(Propylcarbamyl)oxirane-2-carbonyl)-L-isoleucyl-L-proline (Sigma-Aldrich)
Inhibitor Cathepsinu L	Arg-Lys-Leu-Leu-Trp-NH <sub>2</sub> (Sigma-Aldrich)
E-64	L-trans-epoxysuccinyl-leucyl-amido (4-guanidino) butan (Sigma-Aldrich)
DFP	Diisopropylfluorofosfát (Sigma-Aldrich)
PMSF	Phenylmethylsulfonyl fluorid (Sigma-Aldrich)
TPCK	Tolylsulfonylphenylalanyl chloromethyl keton (Sigma-Aldrich)
TLCK	N- -p-tosyl-L-lysine chloromethyl keton (Sigma-Aldrich)
Z-Phe-Arg-FMK	Bachem
Z-Phe-Ala-FMK	Bachem
Z-Phe-Tyr-FMK	Bachem
Elastatinal	Sigma-Aldrich
Calpain inhibitor	N-Acetyl-Leu-Leu-Norleu-al, N-Acetyl-L-leucyl-L-leucyl-L-norleucinal(Sigma-Aldrich)
Aprotinin	Sigma-Aldrich
Trypsin inhibitor	Sigma-Aldrich

### SDS PAGE, Western blot, 2D elektroforéza

DTT - Dithiothreitol	Sigma-Aldrich
Tween 20	Bio-Rad
Bovine Serum Albumins (BSA)	Sigma-Aldrich
Non-Fat Dry Milk	Bio-Rad
5 x vzorkový pufr neredukujúci	2% SDS; 25% glycerol; 0,1 % bromphenol blue; 60 mM Tris/HCl pH 6,8
5 x vzorkový pufr redukujúci	2% SDS; 25% glycerol; 0,1 % bromphenol blue; 60 mM Tris/HCl pH 6,8, 0,1% DTT
10 x Tris/Glycine/SDS pufr (TGS)	Bio-Rad
Blotovací pufr	10 ml 10x TGS; 70 ml H <sub>2</sub> O; 20 ml metanol
PBS-T	0,1M PBS pH 7,2, 0,05% Tween 20
Streptavidin HRP	Sigma
BenchMark™ Protein Ladder	Invitrogen
Precision Plus Protein™ Dual Color Standards	Bio-Rad
Biotínom zna ené DCG 04	Sigma
Poncoeu	1% Poncoeu v H <sub>2</sub> O, Sigma
Roztok CBBR	1,2 g Coomassie brilliant blue; 300 ml metanol; 240 ml H <sub>2</sub> O; 60 ml kys. octová
Odfarbovací roztok	250 ml metanol; 100 ml kys. octová; 650 ml H <sub>2</sub> O
Trypsin inhibitor	Sigma-Aldrich

Rehydratačný pufr - 2D	7M urea; 2M thiourea; 4% CHAPS, 100 mM DTT; 0,5% amfolyty; bromfenol blue
Equilibračný pufr I. - 2D	6M urea; 0,05M Tris-HCl pH 8,8; 2% SDS ; 20% glycerol; 2% DTT
Equilibračný pufr II. - 2D	6M urea; 0,05M Tris-HCl pH 8,8; 2% SDS ; 20% glycerol; 2,5% iodacetamid

## Molekulárne metódy a tvorba rekombinantného proteínu:

### RNA izolácia, DNA elektroforéza, PCR

TRIzol	Invitrogen
50 x TAE buffer	Invitrogen
Agaróza	Invitrogen
EmeraldAmp MAX PCR MM	Takara ó Bio
EmeraldAmp GT PCR MM	Takara ó Bio
HyperLadder IV	Bioline
Syber green	Invitrogen

### Média a i. pre pestovanie baktérií

Low salt LB médium	10 g tryptonu; 5 g kvasničného extraktu; 10 g chloridu sodného; pH 7,0 v 1l sterilnej H <sub>2</sub> O
SOC médium	Invitrogen
LB agar	10 g tryptonu; 5 g kvasničného extraktu; 10 g chloridu sodného; 15 g agaru; pH 7,0 v 1l sterilnej H <sub>2</sub> O
Kanamycín	Sigma-Aldrich
Ampicilín	Sigma-Aldrich
IPTG	Promega
xGal	Promega

### Média a i. pre pestovanie kvasiniek (rekombinantný proteín)

BMG	100mM fosfátový pufr, pH 6, 1,34 % YNB, 4 x 10 <sup>-5</sup> % Biotin, 1% glycerol
BMM	100mM fosfátový pufr, pH 6, 1,34 % YNB, 4 x 10 <sup>-5</sup> % Biotin, 0,5% metanol
YPD	1% kvasničného extraktu, 2% pepton, 2% glukóza, 2% agar, 100 g/ml Zeocin
YPDS	1% kvasničného extraktu, 2% pepton, 2% glukóza, 2% agar, 100 g/ml Zeocin, 1M sorbitol
Zeocín	Sigma-Aldrich
Xho I restriktívna endonukleáza	Promega
Not I restriktívna endonukleáza	Promega
Sac I restriktívna endonukleáza	Promega

## Histologické metódy, TEM, imunolokalizácia

4% paraformaldehyd	4% paraformaldehyd v 0,1M PBS, pH 7,2
Ehrlichov kyslý hematoxilin	2g hematoxilin, 100 ml EtOH, 10 ml kys. octová, 100 ml glycerín 100 ml ddH <sub>2</sub> O, 3g KAl (SO <sub>4</sub> ) <sub>2</sub>
Kakodylátový pufr (TEM)	0,1M kakodylát sodný, 0,1M HCl, 0,1M sacharóza, 1mM CaCl <sub>2</sub> v ddH <sub>2</sub> O
HEPES pufr (TEM)	0,4M HEPES, 0,15M NaCl v ddH <sub>2</sub> O, pH 7,6

## Kity

Opti 4CN substrate kit	Bio-Rad
Quant-IT Protein Assay Kit	Invitrogen
DirectZol RNA Mini Prep	Zymo Research
mRNA Capture Kit	Roche
Transcriptor First Strand cDNA Synthesis Kit	Roche
Zymoclean Gel DNA Recovery Kit	Zymo Research
Topo TA Cloning Kit	Invitrogen
pGEM - T Easy Vector Systems	Promega
High Pure Plasmid Isolation Kit	Roche
Genopure Maxi Plasmid Kit	Roche
EasySelect <i>Pichia</i> Expression Kit	Invitrogen
First Choice RML-RACE Kit	Ambion
JB-4 Plus Embedding Kit	Polysciences, Inc.
Poly/Bed812/Araldite 502	Polysciences, Inc.
Rapid library preparation kit	Roche
Rapid library MID adaptors kit	Roche
Agencourt AMPure XP - PCR Purification	Beckman Coulter

## Pouflité primery

<b>Degenerované primery ( vytvorené na základe aktívnych miest cysteínových peptidáz)</b>	
DP1 forward	5'- CAA GGN CAR TGY GGI TCN TGC TGG - 3'
DP2 forward	5'- CAG GGN CAR TGY GGI TCN TGC TGG - 3'
DP3 forward	5'- CAA GGN CAR TGY GGI TCN TGT TGG - 3'
DP4 forward	5'- CAG GGN CAR TGY GGI TCN TGT TGG - 3'
DP1 reverse	5'- CCA NSA RTT YTT IAC RAT CCA ATA - 3'
DP2 reverse	5'- CCA NST RTT YTT IAC RAT CCA ATA - 3'
DP3 reverse	5'- CCA NSA RTT YTT IAC RAT CCA GTA - 3'
DP4 reverse	5'- CCA NST RTT YTT IAC RAT CCA GTA - 3'
<b>Primery pre RACE PCR</b>	
5'RACE DpCL1 GSP1	5'-GAA ACC AGT GAC CGA AGC AA - 3'
5'RACE DpCL1 GSP2	5'- GGC CTC TCC TGT ATC ACC AGA GAC ATA GGG AT - 3'
5'RACE DpCL1 GSP3	5'- CCG AAA TCA GAG CTG CAA TCC ACA - 3'
5'RACE DpCL2 GSP1	5'- AAT CGA GCG GTA GGA CTT GA - 3'
5'RACE DpCL2 GSP2	5'- GCT TAG CGC GAT ATG GGT AGG CTT GAT CAG TT - 3'
5'RACE DpCL2 GSP3	5'- CCA TCA CAG CCA TTG TTC CCA TCA - 3'
5'RACE DpCL3 GSP1	5'- AGG GCG ATA CGG ATA GGA TT - 3'
5'RACE DpCL3 GSP2	5'- CGA AAG CTG TAC TCA CGA GCC CAC CAT TGC AT - 3'
5'RACE DpCL3 GSP3	5'- AAC GAG TTG TTT GGG CGA TAG GCT - 3'

3'RACE DpCL1	5' - TCA GAT CCC GAG TGT ATG GGT TCA - 3'
3'RACE DpCL2	5' - CGC ATA AAC CAT GGA GTT CTG GCA - 3'
3'RACE DpCL3	5' - CGA TGC ATG CTC CCC AGA CAA TCT - 3'
<b>Expresné primery (pre tvorbu rekombinantného DpCL3)</b>	
EP DpCL3 forward	5' - ATA CTC GAG AAA AGA TCG CCC GAA CAA CCT CAT CCG TTG TGG CAT - 3'
EP DpCL3 reverse	5' - ATG CGG CCG CCT AAT GAT GAT GAT GAT GAT GTT TGA CTA GTG GCA CGC T - 3'
<b>Primery pre overenie kompletných sekvencií DpCL2 a 3</b>	
DpCL2 forward	5' - ATG AAG TTC ATA TTC ATA CTA GTT - 3'
DpCL2 reverse	5' - TTA TTT GAC TAG TGG CAC GCT ACT - 3'
DpCL3 forward	5' - ATG AAG TTC GTA TTC ATA CTA GTT - 3'
DpCL3 reverse	5' - GTA TTT GAC TAG TGG CAC GCT ACT - 3'
<b>Primery -pecifické pre používané vektory (plazmidy)</b>	
M13 forward	5' - TGT AAA ACG ACG GCC AGT -3'
M13 reverse	5' - CAG GAA ACA GCT ATG ACC -3'
AOXI forward	5' - GAC TGG TTC CAA TTG ACA AGC -3'
AOXI reverse	5' - GCA AAT GGC ATT CTG ACA TCC -3'

## Software

Analýza sekvencií

<http://www.ncbi.nlm.nih.gov/>

<http://www.expasy.org/>

<http://www.ebi.ac.uk/Tools/msa/clustalw2/>

Kontrola primerov

<https://eu.idtdna.com/calc/analyzeryzer/default.aspx>

Úprava sekvencií

Geneious 8.1.2.