Practical Course in Cell Biology

conducted at the Department of Applied Genetics and Cell Biology

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AIMS OF THE PRACTICAL COURSE

Dear participants!

In this practical course, you will investigate the expression pattern of the heat shock protein hsp70 by different methods. Heat shock proteins are produced in mammalian cells as a first line of defense against unfavourable environmental conditions such as elevated temperature, UV irradiation, viral infections, oxygen depletion or exposure to heavy metals.

Heat Shock Protein 70 (hsp70) is a cytosolic protein with a molecular mass of 70 kDa. It belongs to the family of molecular chaperones, which are of key importance for the correct folding of newly synthesized cellular proteins. Hence, basal levels of hsp70 are detectable in all mammalian cells. However, transcription of the hsp70 gene is strongly increased upon cellular stress.

Your tasks include:

- Culture of murine connective tissue cells (LTK^- cells)
- Treatment of LTK^- cells with cadmium chloride
- Detection of the newly synthesized hsp70 mRNA by RT-PCR
- Detection of the newly synthesized hsp70 protein by immunoblotting
- Detection of the activity of the hsp70 promoter via expression of the reporter Green Fluorescent Protein (GFP)

Have fun!

Lukas Mach and Georg Seifert

Cell culture

The cell line LTK$^-$ is a thymidine kinase (TK)-minus variant of mouse L cells, one of the first mammalian cell lines established. The TK$^-$ phenotype of the cells has no relevance for the experiments performed in this practical course.

Culture medium: 500 ml DMEM (Dulbecco's Modified Eagle's Medium containing Glutamax as glutamine source)

50 ml FBS (fetal bovine serum; heat-inactivated for 30 minutes at 56°C)

5 ml Penicillin/Streptomycin (10 mg/ml each)

The cultures should be checked daily by microscopy for their confluency, morphology and microbial contaminations.

Passaging of cells

The cells are grown in culture-grade plastic dishes (diameter: 100 mm) in 10 ml culture medium at 37°C in a water-saturated atmosphere with a CO_2 concentration of 5%.

- remove the culture medium by suction, then rinse the cell layer with 10 ml phosphate-buffered saline (PBS)
- add 1 ml 0.25% trypsin (in PBS) dropwise to the cultures
- incubate for 5-10 minutes at 37°C until the cells detach
- stop the reaction by addition of 9 ml culture medium (FBS inhibits trypsin)
- add 7 ml culture medium to each of two new dishes and combine with 3 ml cell suspension
- grow cells at 37°C

Treatment of LTK$^-$ cells with $CdCl_2$

- adjust LTK$^-$ cultures to a final concentration of 100 µM $CdCl_2$

  Stock solution (sterile): 10 mM $CdCl_2$ in double-distilled water (ddW)

- incubate for 3 hours (RNA) or 4 hours (protein) at 37°C
- use cells for the respective experiments

Extraction of cytosolic proteins

To prepare a cytosolic protein extract, mammalian cells are first swollen in a hypoosmotic buffer and then lysed by addition of the non-ionic detergent Nonidet P-40 (NP-40).

- wash cells grown in a 100-mm dish twice with 5 ml PBS prior to overlaying them with 1 ml PBS
- detach the cell layer gently with a cell scraper
- transfer the cell suspension into an Eppendorf tube
- sediment cells by centrifugation for 5 minutes at 1000 x g (3000 rpm)
- resuspend cell pellet in 450 µl protein extraction buffer
- incubate for 10 minutes on ice
- add 50 µl 1% NP-40
- incubate for 15 minutes on ice
- centrifuge sample for 15 minutes at 10000 x g (13000 rpm)
- transfer supernatant into a fresh tube
- store protein extract on ice
- determine the total protein concentration of the extract (see next section)
- dilute sample aliquots (20 µg protein) with ddW to 20 µl, then add 20 µl 2 × SDS-PAGE sample buffer
- store SDS-PAGE samples and residual protein extracts at -20°C

1 × SDS-PAGE sample buffer:	125 mM Tris/HCl, pH 6.8
	10 mM dithioerythritol (DTE)
	1% SDS
	10% glycerol
	0.01% bromophenol blue

protein extraction buffer:	10 mM Tris/HCl, pH 7.5
	10 mM KCl
	0.1 mM EDTA

Quantitation of proteins

The total protein content of cytosolic protein extracts prepared as outlined above is determined by the Bradford method, which is based on the shift of the absorption maximum of Coomassie Brilliant Blue G-250 upon binding to proteins. The protein concentration of the samples is derived from a standard curve generated using bovine serum albumin (BSA).

- prepare a BSA dilution series (0, 1, 2, 3, 4, 5 mg/ml BSA in ddW)
- dilute dye concentrate (Bio-Rad) 1:5 with ddW just prior to use
- add 2 µl sample, standard solution or blank to 1 ml diluted Bio-Rad reagent and mix immediately by vortexing
- use protein extraction buffer supplemented with 0.1% NP-40 as blank for the samples
- incubate for at least 5 minutes at room temperature
- transfer to plastic cuvettes (don't use quartz cuvettes for this assay!)
- measure the optical density (OD) at 595 nm in a spectrophotometer
- generate a standard curve (0-5 mg/ml BSA) using MS-Excel
- calculate the protein content of the extracts

Electrophoretic separation of proteins

Proteins can be separated according to their size by means of polyacrylamide gel electrophoresis (PAGE) in the presence of the denaturing detergent sodium dodecyl sulfate (SDS). Before SDS-PAGE analysis, disulfide bridges between protein subunits are cleaved by reduction with dithioerythritol (DTE). A discontinuous buffer system based on a pH difference between stacking gel and electrophoresis buffer provides for focussing of the protein bands.

- mix components of the separating gel (12.5% acrylamide):

  3.5 ml 30% acrylamide
  1.1 ml 1% bisacrylamide
  2.1 ml 1.5 M Tris/HCl, pH 8.8
  1.6 ml ddW

- degas for 5 minutes using a water-driven vacuum pump
- add 84 µl 10% SDS, 5 µl TEMED und 50 µl 10% ammonium persulfate (APS) in this order under gentle mixing

- pour separating gel using 1.5-mm spacers
- cover with a thin (1-2 mm) layer of isopropanol, then allow to polymerize for 30 minutes
- mix components of the stacking gel (4% acrylamide):

  650 µl 30% acrylamide
  445 µl 1% bisacrylamide
  850 µl 0.5 M Tris/HCl, pH 6.8
  1435 µl ddW

- degas for 5 minutes using a water-driven vacuum pump
- add 35 µl 10% SDS, 2.5 µl TEMED und 25 µl 10% APS in this order under gentle mixing
- pour stacking gel after insertion of a 1.5-mm comb
- allow to polymerize for 30 minutes
- prepare protein electrophoresis buffer (1 liter per electrophoresis tank):

  3 g/l Tris
  14.4 g/l glycine
  1 g/l SDS

- remove comb and rinse sample wells with protein electrophoresis buffer
- thaw samples and controls (provided by the supervisors) and incubate for 5 minutes at 95°C
- dilute marker stock solution (5 µl) with 35 µl 1 × SDS/PAGE sample buffer (no boiling!)
- load wells with markers and samples respectively
- fill all empty wells with 40 µl 1 × SDS-PAGE sample buffer
- perform electrophoresis at 200 V until the tracking dye (bromophenol blue) reaches the bottom of the separating gel (approx. 1 hour)

Detection of proteins by Western blotting

Proteins separated by SDS-PAGE can be transferred electrophoretically onto a nitrocellulose membrane for further analysis. In our case, the membrane is then incubated first with an antibody to hsp70 and then with an antibody to glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Binding of the antibodies to their respective antigens (hsp70 or GAPDH) is detected with a suitable secondary antibody conjugated with horseradish peroxidase (HRP). HRP catalyses the oxidation of luminol in the presence of hydrogen peroxide. This is accompanied by the emission of light, which can be detected using photographic film. The molecular mass of hsp70 is 70 kDa and that of GAPDH 36 kDa.

- prepare Western blotting buffer (1 liter per blotting tank):

  14.4 g/l glycine
  3.03 g/l Tris
  0.37 g/l SDS
  200 ml methanol

- equilibrate separating gel for 5 minutes in Western blotting buffer

- soak membrane and filter paper in Western blotting buffer (use a separate tray)

- assemble a sandwich of sponge, 3 layers of filter paper, gel, membrane, 3 layers of filter paper and sponge following the instructions of the supervisors

- place sandwich and cooling unit in blotting tank

- fill tank with Western blotting buffer

- conduct transfer at 100 V for 1 hour

- remove and disassemble sandwich; discard gel and filter paper but not the sponges

- stain membranes for 5 minutes with 0.2% Ponceau S in 3% trichloroacetic acid (TCA)

- destain with ddW

- take a picture (or scan) of the stained membrane

- wash membrane twice for 5 minutes with PBS to remove the stain

- block membrane with blocking solution overnight at room temperature (shaker)

  blocking solution: 3% BSA in PBS

Immunochemical detection of heat shock protein 70 (hsp70)

- wash blocked membrane twice for 5 minutes with PBS/0.05% Tween 20 (PBST)
- centrifuge primary antibody solution (1 mg/ml mouse monoclonal antibody against hsp70) for 10 minutes at 10000 x g (13000 rpm)
- withdraw 1 µl supernatant
- dilute with 10 ml 3% BSA in PBST (1:10 000 dilution)
- incubate membrane in the primary antibody solution for at least 1.5 hours at room temperature (shaker)
- wash membrane five times for 5 minutes with PBST
- centrifuge secondary antibody (goat antibody against mouse immunoglobulin G, conjugated with HRP) as above
- withdraw 0.5 µl supernatant
- dilute with 10 ml PBST (1:20 000 dilution)
- incubate membrane in the secondary antibody solution for at least 1.5 hours at room temperature (shaker)
- wash membrane five times for 5 minutes with PBST
- rinse membrane twice with PBS
- cover membrane with freshly prepared detection reagent (mix 1.5 ml luminol/enhancer with 1.5 ml peroxide solution) and incubate for 5 minutes
- remove membrane with blunt forceps and drain excess reagent
- seal membrane in a plastic bag
- expose membrane in a cassette to photographic film (suitable exposure times: usually between 10 seconds and 1 minute)
- soak film for 1 minute in regular photographic developer
- wash film for 30 seconds with water
- soak film for 1 minute in regular photographic fixative
- rinse film thoroughly with tap water

Immunochemical detection of glyceraldehyde 3-phosphate dehydrogenase (GAPDH)

- wash membrane after hsp70 detection twice for 5 minutes with PBS
- block membrane again with blocking solution overnight at room temperature (shaker)
- wash blocked membrane twice for 5 minutes with PBST
- centrifuge primary antibody solution (0.2 mg/ml rabbit polyclonal antibody against GAPDH) for 10 minutes at 10000 x g (13000 rpm)
- withdraw 5 µl supernatant
- dilute with 10 ml 3% BSA in PBST (1:2000 dilution)
- incubate membrane in the primary antibody solution for at least 1.5 hours at room temperature (shaker)
- wash membrane five times for 5 minutes with PBST
- centrifuge secondary antibody (goat antibody against rabbit immunoglobulin G, conjugated with HRP) as above
- withdraw 0.5 µl supernatant
- dilute with 10 ml PBST (1:20 000 dilution)
- incubate membrane in the secondary antibody solution for at least 1.5 hours at room temperature (shaker)
- wash membrane five times for 5 minutes with PBST
- rinse membrane twice with PBS
- cover membrane with freshly prepared detection reagent (mix 1.5 ml luminol/enhancer with 1.5 ml peroxide solution) and incubate for 5 minutes
- remove membrane with blunt forceps and drain excess reagent
- seal membrane in a plastic bag
- expose membrane in a cassette to photographic film (suitable exposure times: usually between 10 seconds and 1 minute)
- develop film as outlined above

Isolation of RNA from mammalian cells

The isolation of intact RNA from mammalian tissues and cultured cells is complicated by the presence of ribonucleases (RNAses). Body fluids such as sweat are also rich in these enzymes. It is therefore important to wear gloves and use RNAse-free plasticware throughout the isolation procedure. As far as possible, solutions are pretreated with the RNAse inactivator diethyl pyrocarbonate (DEPC). The extraction buffer („RNA lysis buffer“) contains guanidine isothiocyanate (GTC) to inactivate cellular RNAses. All enzymatic reactions are complemented with RiboLock, a recombinant version of an RNAse-inhibitory protein originally purified from human placenta.

1. remove culture medium by suction

2. add 400 µl RNA lysis buffer (contains GTC) dropwise to the cell layer, then detach cells with a cell scraper

3. draw cell lysate into a 1-ml syringe fitted with a 20-Gauge needle and expel again to reduce the viscosity of the sample (fragmentation of genomic DNA); repeat this step four times

4. transfer lysate into an Eppendorf tube and store on ice

5. remove 175 µl lysate and transfer this aliquot into a fresh Eppendorf tube

6. add 350 µl RNA dilution buffer (blue); mix by pipetting the whole sample up-and-down three times

7. incubate for 3 minutes at 70°C in a heating block

8. centrifuge for 10 minutes at 13000 rpm

9. transfer supernatant into a fresh Eppendorf tube

10. add 200 µl ethanol; mix by up-and-down pipetting of the whole sample (three times)

11. apply sample to a spin column containing silica-coated beads

12. centrifuge for 1 minute at 13000 rpm

13. empty buffer collection tube (bottom part of the column)

14. add 600 µl RNA wash solution to the column

15. centrifuge for 1 minute at 13000 rpm

16. empty buffer collection tube

17. combine 40 µl DNAse buffer (yellow), 5 µl 0.09 M $MnCl_2$ and 5 µl DNAse solution in an Eppendorf tube and mix gently by up-and-down pipetting

18. add mixture dropwise to the column (ensure even distribution)

19. incubate for 15 minutes at room temperature

20. add 200 µl DNAse stop solution to the column

21. centrifuge for 1 minute at 13000 rpm

22. add 600 µl RNA wash solution to the column

23. centrifuge for 1 minute at 13000 rpm

24. empty buffer collection tube

25. add 250 µl RNA wash solution to the column

26. centrifuge for 1 minute at 13000 rpm

27. place column on a fresh Eppendorf tube

28. add 100 µl nuclease-free water to the column (ensure even distribution)

29. incubate for 2 minutes at room temperature

30. centrifuge for 1 minute at 13000 rpm

31. store eluate on ice

Quantitation of RNA

The RNA concentration of the samples is determined by spectrophotometry at 260 nm. At this wavelength, an OD of 1.0 corresponds to an RNA content of 40 µg/ml. Expect an OD_{260}/OD_{280} ratio between 2.1 and 2.3 for RNA isolated by this procedure.

- switch on UV lamp at least 10 minutes prior to measurement
- dilute 5 µl sample with 120 µl TE buffer (1:25 dilution)
- measure OD_{260} and OD_{280} against TE buffer as blank (in a quartz microcuvette)
- calculate the RNA concentration of the undiluted sample: 1.0 OD_{260} (of the 1:25 dilution) = 1.0 µg RNA/µl sample
- calculate OD_{260}/OD_{280} ratio
- remove two aliquots corresponding to 1.0 µg RNA for analysis by gel electrophoresis and RT-PCR; store aliquots and residual sample at -20°C

Electrophoretic analysis of RNA

The purified RNA samples are analysed by electrophoresis to (a) validate their RNA content as determined by spectrophotometry and (b) to assess their quality. Two major bands should be detectable, corresponding to 28S rRNA (4.7 kb) and 18S rRNA (1.9 kb). The intensities of these bands should display a ratio of approx. 2:1. Bands of >10 kb indicate contamination of the samples with genomic DNA.

1. seal gel tray with adhesive tape (one gel is sufficient for the samples of all groups)

2. prepare a 1.0% agarose gel: resuspend 1.0 g agarose in 98 ml ddW and dissolve it in the microwave oven; add 2 ml 50 × TAE buffer und 5 µl ethidium bromide stock solution (5 mg/ml)

3. pour gel and allow it to solidify for 30 minutes at room temperature

4. prepare 1 liter of 1 × TAE buffer (electrophoresis buffer) and fill electrophoresis tank

5. insert tray into electrophoresis tank

6. dilute RNA samples (1.0 µg RNA each) with nuclease-free water to 10 µl; add 2 µl 6 × DNA loading buffer

7. load gel with samples (10 µl) and markers (10 µl of a 1 kb DNA ladder)

8. conduct electrophoresis for approx. 1 hour at 80 V

9. visualize bands by transillumination with UV light (gel documentation system); save image as a TIF file

Reverse transcription of RNA

Messenger RNA (mRNA) can be transcribed into complementary DNA (cDNA) by the enzyme reverse transcriptase (RT) in the presence of a suitable primer, in our case an oligo(dT) primer which binds to all polyadenylated RNAs. cDNA synthesis also requires the presence of the deoxynucleotides dATP, dCTP, dGTP and dTTP. The cDNA thus generated can then be analysed for the presence of selected transcripts by the polymerase chain reaction (PCR).

1. dilute a sample aliquot containing 1.0 µg RNA with nuclease-free water to 11 µl

2. add 1 µl $(dT)_{18}$ primer (0.5 mg/ml)

3. incubate for 5 minutes at 65°C

4. cool for 5 minutes on ice

5. spin for 10 seconds to sediment any condensate

6. add the following reagents in this order:

4 µl	5 × reaction buffer
1 µl	RiboLock RNase Inhibitor (20 U/µl)
2 µl	dNTP mix (10 mM each)
1 µl	RevertAid H Minus M-MuLV RT (200 U/µl)

7. mix by up-and-down pipetting, then spin down quickly

8. incubate for 60 minutes at 42°C, then spin for 10 seconds to sediment any condensate

9. stop reaction by heating in a thermoblock (5 minutes, 70°C)

10. store cDNA samples at -20°C

Polymerase chain reaction

The polymerase chain reaction (PCR) permits the selective detection of a 520-bp fragment of hsp70 cDNA by the use of the thermostable *Taq* DNA polymerase und specific oligonucleotide primers. The quality of the cDNA samples is assessed in parallel by anplification of a 980-bp fragment of GAPDH cDNA. GAPDH, an enzyme involved in glycolysis, is expressed constitutively in most mammalian cells.

1. perform the subsequent steps on ice: mix components in the stated order in 0.2-ml Eppendorf tubes (2 × hsp70, 2 × GAPDH); vortex quickly prior to addition of *Taq* DNA polymerase:

10 µl	nuclease-free water
5 µl	5 × PCR buffer
2.5 µl	forward primer (10 pmol/µl)
2.5 µl	reverse primer (10 pmol/µl)
2.5 µl	dNTP mix (2.5 mM each)
0.5 µl	*Taq* DNA polymerase (5 U/µl)

2. add 2 µl of the respective cDNA solution

3. mix by up-and-down pipetting

4. spin down for 10 seconds

5. place tubes in a PCR thermocycler and start the following programme (duration: approx. 3 hours):

   3 minutes (min) 94°C

   1 min 94°C/1 min 55°C/1 min 72°C (30 cycles)

   4 min 72°C

6. seal gel tray with adhesive tape (two gels are sufficient for the samples of all groups)

7. prepare a 2.0% agarose gel: resuspend 2.0 g agarose in 98 ml ddW and dissolve it in the microwave oven; add 2 ml 50 × TAE buffer und 5 µl ethidium bromide stock solution (5 mg/ml)

8. pour gel and allow it to solidify for 30 minutes at room temperature

9. prepare 1 liter of 1 × TAE buffer (electrophoresis buffer) and fill electrophoresis tank

10. insert tray into electrophoresis tank

11. load gel with samples (10 µl) and markers (10 µl of a 100 bp DNA ladder)

12. conduct electrophoresis for approx. 1 hour at 80 V

13. visualize bands by transillumination with UV light (gel documentation system); save image as a TIF file

Detection of the activity of the hsp70 promoter with Green Fluorescent Protein as reporter

The cell line LHG8 was derived by stable transfection of LTK^- cells with the plasmid phsp70-GFP, which harbours an expression cassette consisting of the hsp70 promoter and the coding region of the Green Fluorescent Protein (GFP) gene. This plasmid also contains the bacterial neomycin resistance gene *neo*^{r} as a selection marker. The latter gene encodes a phosphotransferase capable of detoxifying neomycin as well as its derivative Geneticin (G418), which is toxic for mammalian cells. This can be exploited for the selection of cell lines expressing *neo*^{r}. Hence, LHG8 cells are maintained in regular culture medium (see section *Cell culture*) plus 0.3 mg/ml G418. Upon cellular stress, LHG8 cells synthesize GFP which can be detected by fluorescence microscopy.

- harvest one 100-mm dish LHG8 cells (see *Cell culture*) by trypsinization (gives 10 ml cell suspension)
- dilute 3 ml cell suspension with 6 ml culture medium
- place 2 round cover slips in each of three 35-mm dishes
- add 2 ml prediluted cell suspension to each dish
- incubate overnight at 37°C
- add 5 µl 10 mM $CdCl_2$ to <u>one</u> dish and 10 µl to another (final $CdCl_2$ concentration: 25 and 50 µM, respectively)
- incubate for 18 hours at 37°C
- remove culture medium by suction
- wash cells three times with 2 ml PBS + 0.5 mM $CaCl_2$ + 0.5 mM $MgCl_2$ (PBS + Ca^{2+}, Mg^{2+})
- cover cells gently with 2 ml fixative (4% paraformaldehyde in PBS + Ca^{2+}, Mg^{2+}) and incubate for 10 minutes at room temperature
- wash cells three times with 2 ml PBS + Ca^{2+}, Mg^{2+}
- add 2 ml PBS + Ca^{2+}, Mg^{2+} followed by 20 µl 10 µg/ml 4',6-diamidino-2-phenylindole (DAPI); mix gently
- incubate for 15 minutes at room temperature
- wash cells three times with 2 ml PBS + Ca^{2+}, Mg^{2+}
- place 5 µl mounting medium (Citifluor) in the center of three microscopy slides

- remove one cover slip from each dish and wash it by dipping into ddW (repeat five times)
- drain excess ddW
- place cover slip with the turbid side (cells) facing down on top of the mounting medium
- remove excess mounting medium
- seal cover slip with nail polish
- perform fluorescence microscopy (green fluorescence: GFP; blue fluorescence: DAPI)

QUESTIONNAIRE

PRACTICAL COURSE IN CELL BIOLOGY

Please answer the following questions in the space provided:

1. How is the unwanted activity of RNAses inhibited in the course of molecular biological experiments? Please name the three RNAse-inhibiting reagents used in this course and describe their mode of action and application.

2. Why do stressed LHG8 cells fluoresce? In which subcellular compartments is GFP detected? What is DAPI used for?

3. How can the expression of a given gene be analysed by means of RT-PCR?

4. How is separation according to molecular mass achieved in protein electrophoresis? What is the principle of discontinuous electrophoresis?

All the best!

Lukas MACH and Georg SEIFERT

Instruction for Use

❶ Thaw the ladder at room temperature for a few minutes to dissolve precipitated solids. **DO NOT BOIL!**

❷ Mix gently, but thoroughly, to ensure the solution is homogeneous.

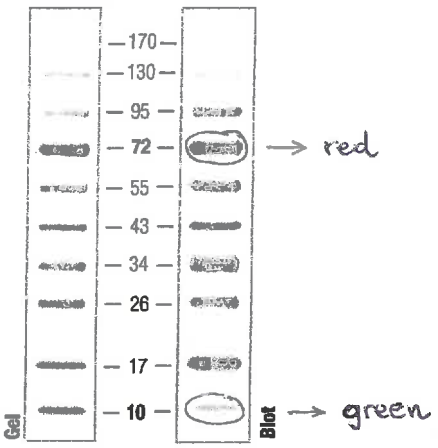
❸ Load the following volumes of the ladder on an SDS-polyacrylamide gel:
– 5 µl per well for mini gel,
– 10 µl per well for large gel.
Use the same volumes for Western blotting.

❹ After the run is complete, stain the gel or perform Western transfer procedure as desired.

Note

- Each lot of the PageRuler™ Prestained Protein Ladder is calibrated against a precisely sized, PageRuler™ Unstained Protein Ladder and calculated apparent molecular weights are reported in the picture.
- For precise molecular weight determinations use PageRuler™ Unstained Protein Ladder, #SM0661, *see* www.fermentas.com.
- In 8 or 10% gels low molecular weight proteins may migrate with the dye front.
- Loading volumes are intended for use in gels with a thickness of 0.75 mm. For thicker gels, the recommended loading volume should be increased.
- PageRuler™ Prestained Protein Ladder could be used in Western blotting with all common membranes: PVDF, nylon and nitrocellulose.
- Longer transfer times or higher transfer voltages may be required for Western blotting of large (>100 kDa) proteins.

Lot specific MW, kDa



4-20% Tris-glycine SDS-PAGE

IMPROVEMENT
Green 10 kDa reference band for easier orientation.

continued on back page

RECOMMENDATIONS FOR USE

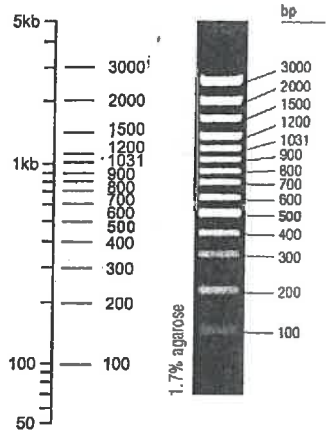
- Prepare GeneRuler™ 100bp DNA Ladder Plus before loading as following:
  1µl (0.5µg) of GeneRuler™ 100bp DNA Ladder Plus
  1µl of 6x Loading Dye Solution
  4µl of deionised water
- Vortex gently just prior to use.
- Do not heat before loading.
- Apply 0.1µg (0.2µl) of DNA Ladder per 1mm of agarose gel lane width.
- Following electrophoretic separation on agarose gel the DNA bands can be visualised by ethidium bromide staining.
- 1µg of the GeneRuler™ 100bp DNA Ladder Plus contains 102ng (10%) of the 500bp fragment.

PRODUCT USE LIMITATION.
This product is developed, designed and sold exclusively *for research purposes and in vitro use only*. The product was not tested for use in diagnosis or for drug development, nor it is suitable for administration to humans or animals.

Updated March 11, 2000

GeneRuler™
100bp DNA Ladder Plus

Fragment Sizes



RECOMMENDATIONS FOR USE

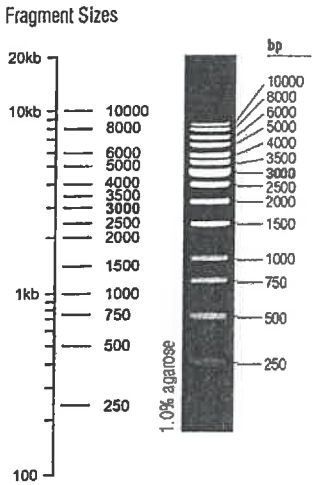
- Vortex gently just prior to use.
- Do not heat before loading.
- Apply 0.1µg (1µl) of DNA Ladder per 1mm of agarose gel lane width.
- Following electrophoretic separation on agarose gel the DNA bands can be visualized by ethidium bromide staining.
- 1µg of the GeneRuler™ 1kb DNA Ladder, ready-to-use, contains ≅310ng (≅31%) of the 3000bp fragment.

PRODUCT USE LIMITATION.
This product is developed, designed and sold exclusively *for research purposes and in vitro use only*. The product was not tested for use in diagnosis or for drug development, nor it is suitable for administration to humans or animals.

Updated March 11, 2000.

GeneRuler™
1kb DNA Ladder,
ready-to-use

Fragment Sizes



SCHEDULE FOR PRACTICAL COURSE IN CELL BIOLOGY

MONDAY	TUESDAY	WEDNESDAY
Cell culture (1 h) Introductory lecture (1 h) Protein extraction (2 h) Protein quantitation (2 h)	Pouring of SDS-PAGE gels (3 h) SDS-PAGE analysis (2 h) Western blotting (2 h) Ponceau staining	Cell culture (2 h) RNA extraction (2 h) RNA quantitation (1 h) Immunochemical detection (part I)

THURSDAY	FRIDAY
RNA electrophoresis (2 h) Reverse transcription (3 h) PCR and DNA electrophoresis (2 h) Immunochemical detection (part II)	Microscopy (2 h)