Eppendorf Thermomixer comfort/compact Eppendorf ThermoStat plus

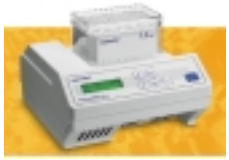
Thermomixer comfort –heating, mixing, cooling–

Temp. control range: (RT –13 °C) to 99 °C [MTPs: (RT –10 °C) to 70 °C], Minimum temp. 1 °C
Incubating accuracy: +/–0.5 °C in the range 20 °C to 45 °C; +/–2 °C in rest of range
Heating-up speed: approx. 5 °C/min.
Cooling-down rate: 2 °C–3 °C/min. from RT to 13 °C below RT 0.5 °C to 1 °C/min.
Mixing: 300–1400 rpm (1500 rpm with 0.5 ml thermoblock); mixing stroke: 3 mm (orbital)
Programming: 2 different program sequences, interval mixing possible in each case
Thermoblocks: 24 x 0.5 ml; 24 x 1.5 ml; 24 x 2.0 ml; MTP + deep-well plates; adapter plate for PCR MTP; tubes 2–5 ml with diameter: 11–11.9 mm, height: 30–76 mm
24 x 1.5 ml–2 ml cryo tubes; 4 x 50 ml Falcon® tubes; 8 x 15 ml Falcon® tubes

Thermomixer compact –heating and mixing–

Temp. control range: (RT + 4 °C) to 99 °C
Incubating accuracy: +/–1 °C in the range up to 45 °C; +/–2 °C in rest of range
Heating-up rate: approx. 5 °C/min.
Mixing: 300 rpm – 1400 rpm; mixing stroke: 3 mm (orbital)
Fixed block: 24 x 1.5 ml

ThermoStat plus –cooling and heating–



Temp. control range: –5 °C to 99 °C (CombiBox up to 95 °C)
Incubating accuracy: +/–0.5 °C in the range –5 °C to 45 °C; +/–2 °C in rest of range
Heating-up rate: approx. 4 °C/min.
Cooling-down rate: 2 °C/min. between 99 °C and 25 °C; 1 °C/min. between 25 °C and –5 °C
Programming: 4 consecutive different temperature-control and time phases possible
Thermoblocks: 24 x 0.5 ml; 24 x 1.5 ml; 24 x 2,0 ml; MTP + deep-well plates; adapter plate for PCR MTP; CombiBox; tubes with diameter: 11–11.9 mm, height: 30–76 mm; 24 x 1.5 ml–2 ml cryo tubes; 4 x 50 ml Falcon® tubes; 8 x 15 ml Falcon® tubes

Product	Thermomixer comfort	Thermomixer compact	ThermoStat plus
Feature	● heats ● cools ● mixes	● heats ● mixes	● heats ● cools
Temperature control range	(RT –13 °C) to 99 °C (MTPs: (RT –10 °C) to 70 °C)	(RT + 4 °C) to 99 °C	–5 °C to 99 °C (CombiBox to 95 °C)
Applications	● Culture of bacteria/yeasts ● Bead technology ● Immunoprecipitation ● Enzyme reactions ● Transformation (in conjunction with the IsoTherm system) ● Denaturing of DNA, RNA, Proteins	● Culture of bacteria/yeasts ● Bead technology at temp. > RT + 4 °C ● Enzyme reactions at temp. > RT + 4 °C ● Transformation (in conjunction with the IsoTherm system) ● Denaturing of DNA, RNA, Proteins	● Maintaining chemicals at a certain temperature ● Enzyme reactions ● Transformation (in conjunction with the IsoTherm system) ● Denaturing of DNA, RNA, Proteins

Gene expression analysis

RNA isolation

Application for **Thermomixer comfort/Thermomixer compact**
Principle of bead technology:
Molecules of a specific identity are immobilized on a specific substrate (bead). The technology is based on binding a specific molecule to the immobilized samples. Binding can be based on either a hybridization or an Ag/Ab reaction. Gentle mixing of the sample improves binding efficiency.

● **Dynabeads Oligo (dT)25 from Dynal Biotech**

Basic principle: Binding PolyA$^+$ RNA to oligo-dT-nucleotides of about 20 bases which are bound to the beads.
Method: Binding the PolyA$^+$ RNA to the beads whilst mixing the sample.

Reverse transcription

Application for **ThermoStat plus**/Thermomixer compact/Thermomixer comfort
Basic principle: Synthesis of cDNA (complementary DNA) from mRNA by means of reverse transcription.
Method: Incubate the reaction at a variety of temperatures
Denaturation of RNA and Primer: e.g. at 70 °C for 10 min
cDNA synthesis: incubate the reaction at 37 °C – 42 °C for 1 h
Stop the reaction by means of heat deactivation, e.g. at 70 °C for 30 min

Microarray hybridization

Application for **Thermomixer comfort/Thermomixer compact**
Basic principle: Molecules of a specific identity are immobilized on a specific substrate (slide). The technology is based on binding a specific molecule to the immobilized samples.
Binding is based on a hybridization reaction.
Mixing the sample improves binding efficiency.

Immunological methods and protein detection

Cell lysis

Application for **ThermoStat plus**/Thermomixer comfort
Basic principle: Cells are lysed in a special lysis buffer (detergent).
Method: Incubate the reaction at 4 °C for 1 h

Immunoprecipitation

Application for **Thermomixer comfort**
Basic principle: A certain protein in solution can be precipitated out of that solution by addition of the appropriate monoclonal antibody and Protein A or Protein G.
Gentle mixing of the sample improves the efficiency of binding.
Method: Incubation at 4 °C for 1 h – 2 h

In vitro translation

Application for **ThermoStat plus**/Thermomixer comfort
Basic principle: *In vitro* translation is understood to be the cell-free synthesis of proteins.
The method for this process can be divided into three parts as outlined below.

- Generation of template DNA which codes for a protein
  For this purpose, cDNA is usually cloned in a vector with SP6, T3 or T7 RNA polymerase promoter.
- This template is used to synthesize RNA by means of *in vitro* transcription.
  Method: The linearized template is transcribed into RNA by means of an RNA Polymerase
  Incubation at 37 °C – 42 °C für 1 h
- Translation of the RNA
  Protein synthesis performed in a eukaryotic translation system
  (wheat germ extract or reticulocyte lysate)
  Method: Incubation at 30 °C for 1 h

Isolation/Purification of Proteins using bead technology

Application for **Thermomixer comfort**
Principle of bead technology
Molecules of a specific identity are immobilized on a specific substrate (bead). The technology is based on binding a specific molecule to the immobilized samples. Binding can take the form of either a hybridization or an Ag/Ab reaction. Gentle mixing of the sample improves binding efficiency.

- **Dynabeads for Protein Isolation from Dynal Biotech**

Basic principle: Binding the protein to the bead-coupled antibody.
Method: - precoating the beads: at 2 °C – 8 °C for 2 h – 24 h
- binding reaction: at 2 °C – 8 °C for 10 min – 60 min

Cloning DNA fragments

This genetic engineering method allows the genetic material from cells or organisms to be modified at will and in a specific manner. To this end, a DNA fragment is linked to a so-called vector, which for example facilitates mass replication of this DNA in a host cell (production of recombinant DNA), and is then transferred to a host cell.
In addition to replication, other interesting properties can be induced by the use of certain vectors – e.g. RNA polymerase promoters in a vector allow straightforward *in vitro* transcription of RNA, whilst viral promoters in the construct enable cDNAs to be expressed in the cells of mammals.

Basic principle:
- Preparation of insert vector DNA
- Ligation of vector and DNA insert
- Transformation into host cell

Preparation of insert DNA and vector

Restriction digest:

Application for **ThermoStat Plus**/Thermomixer compact/Thermomixer comfort
Restriction enzymes detect a specific base sequence of 4, 6 or 8 bases in a DNA double helix and cut both strands of the helix at specific points. Using restriction enzymes, DNA molecules can be cut into specific fragments which are then much easier to analyze and manipulate than the original molecule.
Almost all enzymes generate fragments with one 5'-phosphate end and one 3'-OH end (important for ligation). The ends are either smooth (blunt ends) or overlapping (sticky ends) – if the 5' end is longer, we refer to a 5' overhang, otherwise to a 3' overhang.

Example:
The circular 5.1 kb-long DNA double helix of the tumor-inducing SV40 virus is cut by *Eco*RI at one point, by *Hpal* at 4 points and by *Hind*III at eleven points. A DNA fragment created by a restriction enzyme can still be cut specifically into smaller fragments using another restriction enzyme.
The pattern of these fragments can serve as the *fingerprint* of a DNA molecule.
Small differences between similar DNA molecules are easy to detect, as their restriction fragments can be separated and rendered visible by gel electrophoresis.
A restriction fragment with a particular base sequence can be identified by being hybridized with a marked, complementary DNA strand (Southern Blot).

Method: Incubate the reaction at a specific temperature, e.g. at 37 °C for 1 h.
Stop the reaction by deactivating the enzyme with heat (e.g. at 65 °C for 20 min) or by adding 0.5 M EDTA.

Conversion sticky ends into blunt ends

Application for **ThermoStat plus**/Thermomixer compact/Thermomixer comfort
In general, it is difficult to find suitable restriction cut sites for cloning. Following restriction digest, the DNA fragment is ligated to a vector previously cut with the same restriction enzyme. If the vector envisaged for cloning does not have the appropriate cut site, a different strategy will need to be selected.
The overhanging ends of the fragments can be smoothed off and the fragment then ligated into a vector with smooth, dephosphorylated ends.
The overlapping ends can be filled with a normal Polymerase reaction using a T4 DNA Polymerase (or a Klenow fragment).

Method: Incubate the reaction at a specific temperature,
e.g. at room temperature for 20 min or at 37 °C for 5 min
Stop the reaction by deactivating the enzyme with heat (e.g. at 75 °C for 10 min).

Phosphorylation/Kinasing

Application for **ThermoStat plus**/Thermomixer compact/Thermomixer comfort
Transfer of phosphoryl groups is a fundamental biochemistry reaction. An enzyme which transfers a phosphoryl group from ATP to an acceptor is generally called a kinase.
Potential application:
Production of synthetic, cohesive ends on any DNA molecules.
For this purpose, short, chemically-synthesized DNA linkers are linked to the ends of a DNA fragment or Vector and then cut by restriction digest.

Principle: The 5' end of a decamer linker and that of a DNA molecule are phosphorylated by a polynucleotide kinase and then joined using a ligase from among the T4 phages (link blunt ends of a double helix). Cohesive ends are obtained by cutting open the joined end segments with a suitable restriction enzyme.

Method: Incubate the reaction at a specific temperature, e.g. at 37 °C for 0.5 – 1 h
Stop the reaction by heat deactivation, e.g. at 70 °C for 30 min

Dephosphorylation

Application for **ThermoStat plus**/Thermomixer compact/Thermomixer comfort
If a Vector is cut with just one restriction enzyme, two compatible ends are formed which can be religated with one another. Self-ligation of the Vector is in direct competition with DNA/Vector ligation. This problem can be reduced by dephosphorylating the Vector DNA (CIPen).

Principle: Following restriction digest, phosphate radicals required for ligation remain on the 5' ends of the DNA fragments. If these are removed on the Vector with a phosphatase, no more self-ligation takes place. The DNA fragment which you want to insert in the Vector, on the other hand, still has both phosphate radicals and can therefore still ligate with the Vector DNA.

Method: Incubate the reaction at a specific temperature, e.g. at 37 °C for 1 h.
Stop the reaction, e.g. by adding EDTA (adjusting to 5 mM, pH 8) and by heat deactivation, e.g. at 75 °C for 10 min or at 65 °C for 1 h.

Ligation of Vector and insert DNA

Application for **ThermoStat plus**/Thermomixer comfort
Constructing new DNA molecules in the lab
The Vector used in recombination experiments can be prepared for splicing by being cut open with a restriction enzyme at a single specific point. If an enzyme with overlapping cut sites is used, complementary single-strand ends form which have a specific affinity for one another and are therefore called cohesive ends. Any DNA fragment can be incorporated in this plasmid if it has the same cohesive ends. In order to produce a fragment of this type from a relatively large piece of DNA, it is necessary only to use the same restriction enzyme which was used to open the plasmid DNA.
DNA fragments and open plasmid can be hooked up by means of base pairing and linked by DNA ligase which catalyses the binding of a phosphodiester bond between two DNA chains.
DNA ligase requires a free OH group at the 3' end of one DNA chain and a phosphate group at the 5' end of the other. This reaction is called ligation.

Method: Incubate the reaction at a specific temperature, e.g. at 16 °C for 1 h – 4 h or at 12 °C overnight.

Transformation

Application for **ThermoStat plus + IsoTherm System/Thermomixer comfort/compact + IsoTherm System**
Designing a recombinant molecule
The DNA fragment of interest is bonded covalently to a DNA vector. The key property of a vector consists of replicating autonomously in a suitable host. Plasmids and Phage I, for example, are the vectors of choice for cloning in *E.coli*.
Transfer to the host cell/Transformation
Many cells of bacteria and eukaryotes absorb naked DNA molecules from the medium. Although the degree of efficiency is low (at about one in 10^6 DNA molecules), it is possible to transform a considerable number of cells in this way under suitable experimental conditions.
Method: Incubate the reaction at a variety of temperatures
e.g. on ice/IsoTherm System for 45 min; at 37 °C for 5 min (or at 43 °C for 3 min)/ThermoStat plus; on ice/IsoTherm System for 5 min
Before being plated onto agar, bacteria are shaken at high speed at 37 °C for approx.1 h in order to build up their resistance to antibiotics, for example.

cDNA libraries:

Application for **ThermoStat plus/Thermomixer compact/Thermomixer comfort**
RNA isolation: Thermomixer comfort/Thermomixer compact
Binding PolyA$^+$ RNA to oligo-dT-nucleotides of some 20 bases bound to a matrix. The matrix can consist of cellulose, magnetic beads or latex beads.
E.g. Dynabeads Oligo (dT)25 : bind PolyA$^+$ RNA to the beads whilst mixing the sample

Reverse transcription: ThermoStat plus/Thermomixer compact/Thermomixer comfort
Synthesis of cDNA from mRNA by reverse transcription
Method: Incubate the reaction at a certain temperature, e.g. at 42 °C for 1 h
Stop the reaction by means of heat deactivation, e.g. at 70 °C for 30 min

2-strand synthesis: ThermoStat plus/Thermomixer comfort
using DNA Polymerase I from *E.coli*.
Method: Incubate the reaction at a specific temperature, e.g. at 16 °C for 2 h
Stop the reaction by adding EDTA/glycogen
➢ clonable ds DNA

Culture of organisms

Culture of bacteria in 2 ml Eppendorf tubes

Application for **Thermomixer compact/Thermomixer comfort**
Applications in this area have already arisen in conjunction with LidBac (www.eppendorf.de/applications)
Method: Incubate the culture at 37 °C, 1400 rpm, overnight

Culture of yeasts in 2 ml Eppendorf tubes

Application for **Thermomixer compact/Thermomixer comfort**
Method: Incubate the culture at 30 °C, 1400 rpm, overnight

Radioactive/non-radioactive labeling of DNA

Methods for preparing labeled probes

Nick translation

Application for **ThermoStat plus**/Thermomixer comfort

Principle: Template DNA is partly digested with DNase in the presence of Mg^{2+} ions. Under these conditions, the enzyme cuts only one of the two strands so that the double strand structure is retained. In a second step, the template is processed with DNA Polymerase in the presence of nucleotides, one of which is marked. The polymerase detects the cuts and at that point extends the free 3' end whilst simultaneously breaking down the 5' end.

Method: Incubate at 12 °C – 15 °C for 15 min – 45 min
Inactivate: at 70 °C for 10 min

Random priming

Application for **ThermoStat plus + IsoTherm System**/Thermomixer compact/comfort + IsoTherm System

Principle: Denaturing double-stranded template DNA, then hybridizing with random primers which then serve as primers for a DNA Polymerase (e.g. Klenow fragment). Marking is effected by incorporating either radioactively or non-radioactively marked nucleotides.

Method: Denaturation: at 95 °C for 3 min
Cool on ice/IsoTherm System for 3 min
Incubate at 37 °C/room temperature for 1 h – 2 h.
Inactivate at 70 °C for 10 min

5' labeling with polynucleotide kinase

Application for **ThermoStat plus**/Thermomixer compact/Thermomixer comfort

Principle: Labeling is effected by replacing the phosphate radical on the 5' end with a radioactive phosphate.

Method: **1. Dephosphorylation**
Incubate the reaction at a specific temperature, e.g. at 37 °C for 1 h
Stop the reaction, e.g. by means of heat deactivation, e.g. at 75 °C for 10 min/65 °C for 1 h
2. Marking with [γ-^{32}P]-ATP
Incubate the reaction at 37 °C for 10 min – 30 min
Stop the reaction using 0.5 M EDTA

3' labeling with terminal transferase

Application for **ThermoStat plus**/Thermomixer compact/Thermomixer comfort

Principle: Labeling is effected by terminal deoxynucleotidyl transferase which non-specifically adds deoxynucleotides to the 3' end of single and double-stranded DNA.

Method: Incubate the reaction at 37 °C for 30 min
Stop the reaction: at 70 °C for 10 min

DNA purification

Preparation of genomic DNA

Application for **Thermomixer comfort/Thermomixer compact**

- **Eppendorf gDNA Blood Kit:**
  Proteinase K digest
  Method: Incubate the reaction batch at 70 °C, 900 rpm for 10 min
- **Solubilize genomic DNA** following phenol/chloroform purification and EtOH precipitation
  Method: Slow process which takes 1 – 2 days.
  Can be speeded up with careful movement (300 rpm) at room temperature or 65 °C.

Isolating DNA fragments from agarose gels

Application for **Thermomixer comfort/Thermomixer compact**

- **Eppendorf Perfectprep Gel Cleanup** (centrifugation method)
  Dissolve the piece of agarose gel
  Method: mix the batch at 50 °C, 1000 rpm for 5 min to 10 min
- **Using "glass milk" (silica material)**
  Glass binds DNA in the presence of high concentrations of chaotropic salts and can be eluted again following a washing step with salt/ethanol buffer (low salt concentration)
  Method: – Add high-salt buffer to the piece of agarose and mix the batch at 50 °C, 1000 rpm for 5 min
  – Add "glass milk" and incubate at room temperature for 5 min (gentle mixing improves the efficiency of binding)
  – Centrifuge, wash, centrifuge, dry, elute

Purification of biotinylated samples using bead technology

Application for **Thermomixer comfort/Thermomixer compact**

Principle of bead technology

Molecules of a specific identity are immobilized on a specific substrate (bead). The technology is based on binding a specific molecule to the immobilized samples. Binding can take the form of either a hybridization or an Ag/Ab reaction. Gentle mixing of the sample improves binding efficiency

- **Dynabeads Streptavidin from Dynal Biotech**

Basic principle: Binding the biotinylated DNA to the beads
Method: – Mix the reaction at room temperature for approx. 10 min

Plasmid DNA purification

- **Eppendorf Perfectprep Plasmid Kit**

Application for **ThermoStat plus**/Thermomixer compact/Thermomixer comfort

Method: Heating up the elution buffer
A better yield of Plasmid DNA can be achieved if the elution buffer is heated to 65 °C for Plasmid DNA < 30 kb or to 85 °C for Plasmid DNA > 30 kb.

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