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# THERMOTOOLS Cloning Kit Ready to Use

16 rxns

Store at appropriate temperatures

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## *Description*

Thermotools cloning kit has been designed for cloning and transformation in *Thermus thermophilus*, and can be used for thermostabilisation and expression studies of thermophiles using *Thermus thermophilus* as a model organism. Transformation efficiencies are approximately  $10^7$  transformed colonies / ng plasmid (depending on the plasmid sequence). Thermotools kit components are transported at 4°C. On receipt of this kit each component should be stored as explained below.

The kit contains the following reagents:

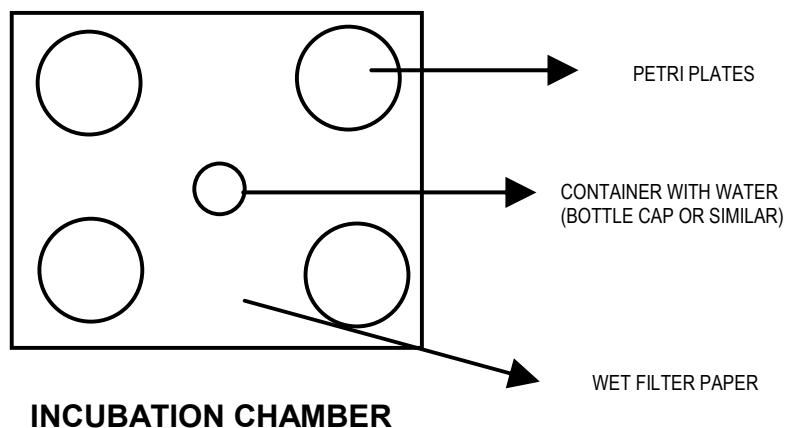
- *Thermus thermophilus* HB27 competent cells. 16 aliquots of bacteria are contained in the kit. Cells retain competency at 4°C and can be stored for a period of 6 months at this temperature. For longer storage keep the bacteria at -20°C. BIOTOOLS recommends storing bacteria for immediate use at 4°C and the remaining aliquots at -20°C
- 250 ml of Transformation Medium. Store at 4 °C. Pre-heat at 70 °C prior to use.
- Petri plates containing special medium + kanamycin 30 mg/ml to improve growth of transformants at 65 °C – 75 °C (optimal temperature is 70 °C). 16 plates. Store at 4°C and protect from light. When stored properly, kanamycin/agar plates are stable for one month. Pre-heat at 70 °C prior to use.
- Plasmid pMK18 is a recombinant plasmid containing sequences from both *T. thermophilus* HB27 and *E. coli* derived pUC18. pMK18 contains replications of origin from both *E. coli* and *T. thermophilus*, and can be used as a shuttle vector between both bacteria. Therefore pMK18 serves as a model system for thermostabilisation projects involving transformation in both organisms. The plasmid contains the *kat* cassette for kanamycine selection, which is active at 37 °C and at 70 °C. The promoter under which the expression of *kat* is directed is a shuttle promoter, and is active in *E. coli* cultures and in *Thermus thermophilus* cultures. DNA concentration: 100 ng/μl. The plasmid should be stored at -20 °C. (Reference: [Plasmid](#), Volume 42, Number 3, November 1999 , pp. 241-245(5))

## *Protocol*

- 1.- Add 1 ml of Transformation Medium to 1 pellet of competent cells. Resuspend thoroughly by gentle pipetting.
- 2.- Transfer to a 10 ml vial (plastic or glass). Incubate 30 min at 70 °C.
- 3.- Add 5 to 7,5 μl of pMK18 (obtained from HB27) to the 10 ml vial.
- 4.- Incubate 4-6 hours at 70 °C. For a long incubation, use of PEG rather than water in the incubation bath is recommended.
- 5.- Centrifuge 2 min at 3,000 rpm. Discard supernatant, leaving 100-200 μl.
- 6.- Plate 100-200 μl in on the provided Petri plates. Begin growth of the kanamycin resistant transformants as described in the following step.

7.- Place a piece of slightly wet filter paper at the bottom of the “Tupperware” container in which the kit is delivered. In the middle of the same container, place a small beaker filled with water (for example, a 4 to 5 cm diameter bottle cap). Humidity in incubation chamber avoids drying of the plates. Plates must be placed face-down to prevent agar drying. Keep container closed and completely sealed during incubation.

8.- Transformants are observed after 2 to 4 days of incubation at 70 °C. For incubations at 65 °C, longer times may be necessary.



<b>Cat. No.</b>	<b>Product</b>
31.117	Thermotools cloning kit Ready to Use (16 rxns)