Thermus thermophilus Growth and Transformation Kit

Product Description

Name: PMK18 Thermus thermophilus Growth and Transformation Kit
Catalog Number: FQ1301 16 rxns

Storage: at appropriate temperatures. On receipt of this kit each component should be stored (see Contains).

Description

Thermus thermophilus Growth and Transformation kit has been designed for cloning and transformation in both E. coli and Thermus thermophilus. The kit can be used for thermostabilisation and expression studies using T. thermophilus as a model organism.

Contains

The kit contains the following components:

- Thermus thermophilus HB27:nar competent cells. 16 aliquots of bacteria are contained in the kit. Competent cells can be stored at 4°C for a period of 6 months; for a longer storage keep them at -20°C. In any case, avoid cycles of freeze-thaw as they reduce cell viability.

- 250 ml of Transformation Medium. Store at 4 ºC and pre-heat at 70 ºC before use.

- 16 Petri Plates with solid selection medium containing special medium + 30 mg/ml kanamycin to improve the growth of transformants at 65 ºC – 75 ºC (optimal temperature 70 ºC). Store at 4ºC protected from light. In these conditions kanamycin/agar plates are stable for a month. Pre-heat at 70 ºC before use.

- 150 ng/µl Plasmid pMKE2 (Moreno R. et al., 2005) contains single sites for the oriented cloning of the coding sequence of external genes, either fused or not to N and/or C-terminal polyhistidine tags. It is a bifunctional E. coli-thermus sp vector, under the control of Pnar promoter. The Pnar promoter, activated by nitrate and anoxia, controls the expression of the nitrate reductase operon K (nar operon), a key element for growth under anaerobic conditions. The ability to grow under anaerobic conditions is encoded within the portable element, nar operon, which can be transferred by cloning in pMKE2. Overproduction of thermozymes may be specifically induced through the combination of nitrate and anoxia.

- An Incubation Chamber for bacterial growth at high temperatures.

The strain T. thermophilus HB27:nar is a derivative of the high transformation efficiency HB27 strain that contains the nar cluster (Ramirez-Arcos et al., 1998). This cluster includes genes for anaerobic respiration on nitrate and the transcription factors required to sense the absence of oxygen and the presence of nitrate. When both signals are present, the promoter of the pMKE2 is induced.

Instruction for use

Cell Growth

1.- Add 1 ml of Transformation Medium to one of the HB27:nar aliquots, resuspend cells carefully and transfer the content to a 100 ml flask containing 20 ml of TB. Incubate in an orbital shaker (150 rpm) at 70 ºC.

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2.- When cells reach an OD550 of 0.4-0.6, separate the culture in 1 ml aliquots in preheated 10-12 ml tubes. Add the plasmid (1-100 ng) and keep the incubation under shaking for 3 hours.

3.- Plate the cells in pre-heated Petri Plates containing 30 mg/I kanamycin. Initiate selection of the kanomycin-resistant transformants as described in the following step.

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

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

**Induction**

1.- Inoculate the transformed cells in the required volume of Transformation Medium containing 30 mg/ml kanamycin, always keeping a 1:5 ratio to the flask volume. Incubate in orbital shaking (150 rpm) at 70 ºC until the cells reach an OD550 of 0.3.

2.- Stop the shaker and add 1:50 of the inducer (2M KNO3), continue the incubation at 70 ºC for 4 hours before harvesting the cell for further analysis.

3.- Take a sample (200 µl) from the supernatant to check the production of nitrite by adding 200 µl of Solution I (0.02 % of N-(1-naphthyl)-ethyl-enediamine in water) and 200 µl of Solution II (1 % of sulphainilamide in 2.5M HCl). A strong pink color after 10 min at RT indicates that the Pnar promoter has been successfully induced.

2M KNO3, Solution I and Solution II are not included in the kit.

**References**


**Related Products**

1. Plasmid pKT1 (100 ng/µl), S54830
2. Plasmid pMK18 (100 ng/µl), S54840
3. Plasmid pMKE1 (150 ng/µl), FQ1310
4. Plasmid pMKE2 (150 ng/µl), BP7010
5. E. coli & Thermus Cloning and Expression Kit, S54851

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Ordering information

Catalog size quantities and prices may be found at [http://www.interchim.com](http://www.interchim.com).
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