



THERMUS THERMOPHILUS GROWTH AND TRANSFORMATION KIT READY TO USE

Store at 4°C

The strain *T. thermophilus* HB27:*nar* is a derivative of the high transformation efficiency HB27 strain that contains the *nar* cluster (Ramírez-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 included in plasmid pMKE2 is induced.

The kit contains the following reagents:

- *Thermus thermophilus* HB27:*nar* 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 (within one month) 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 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.

Cell Growth

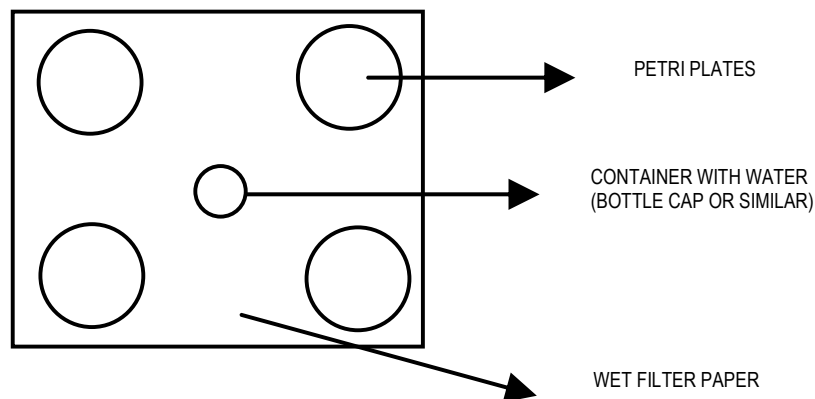
1.- Add 1 ml of Th medium to one of the cell pellets, resuspend the cells carefully, and transfer the content to a 100 mL flask containing 20 ml of TB. Incubate under orbital shaking (150 rpm) at 70 °C.

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. Plasmid pMKE2 is at a concentration of 150 ng/μl.

3.- Plate the cells in pre-heated Th plates containing 30 mg/L of kanamycin. Initiate selection of the kanamycin-resistant transformants as described in the following step.

4.- 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. See diagram.

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



Induction

- 1.- Inoculate the transformed cells in the required volume of TB containing kanamycin (30 mg/L), always keeping a 1:5 ratio to the flask volume. Incubate at 70 °C under shaking as above (150 rpm), until the cells reach an OD₅₅₀ of 0.3.
- 2.- Stop the shaker and add 1:50 of the inducer (KNO₃ 2M), and keep the incubation at 70 °C 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 sulphanilamide in HCl 2.5 M). A strong pink color after 10 min at RT indicates that the Pnar promoter has been successfully induced.

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

Cat No	Product
31.121	<i>Thermus thermophilus</i> Growth and Transformation Kit Ready to use
31.119	Plasmid pMKE1, 150 ng/µl
31.120	Plasmid pMKE2, 150 ng/µl

Ed. 05 November 07