

## 86494 'Terrific Broth', modified (Tartoff-Hobbs Broth, modified)

'Terrific Broth', modified is used with glycerol in cultivation of recombinant *E. coli* strains. Supports higher cell densities and a longer period of log phase growth compared to LB Broth, and therefore usually increases yields of plasmid DNA and recombinant proteins.

### Composition:

Ingredients	Grams/Litre
Protein hydrolyzate NZ-amine	12.0
Yeast extract	23.9
Dipotassium hydrogen phosphate	9.4
Potassium dihydrogen phosphate	2.2

Store prepared media below 8°C, protected from direct light. Store dehydrated powder, in a dry place, in tightly-sealed containers at 2-25°C.

### Directions:

Suspend 47.5 g in 900 ml distilled water. Add 8 ml glycerol (Fluka 49769) and sufficient distilled water to bring the volume to 1 litre. Autoclave at 121°C for 20 minutes.

*Preparation of Working Stock:* Cells from one colony of *E. coli* were transferred to 50 ml of sterile Terrific Broth (modified) in a 250 ml shake flask. The flask was shaken overnight at 37 °C. The following morning the cells were centrifuged at 800 X g for 10 minutes. The supernatant was discarded and the cell pellet was resuspended in 20 ml of fresh Terrific Broth (modified). This working stock can be used immediately or divided into aliquots in sterile cryogenic freezing vials and stored at -70 °C.

*Preparation of Starter culture:* Add 0.3 ml of working stock to 50 ml of Terrific Broth (modified) in a 250 ml shake flask. The flask is shaken overnight at 300 rpm at 37 °C. 1.0 ml of starter culture is immediately transferred to 50 ml of fresh Terrific Broth (modified) in a 250 ml shake flask. The cells are shaken overnight at 300 rpm at 37 °C and the OD<sub>640</sub> is measured in 2 hour increments. The OD<sub>640</sub> for *E. coli* is around 12.0 within eight hours. The OD<sub>640</sub> for *B. subtilis* is about 8.0 within eight hours.

### Principle and Interpretation:

Protein hydrolyzate NZ-amine and yeast extract provide essential nutrients and cofactors for excellent growth of recombinant strains of *E. coli*. The higher yeast extract concentration allows an improved yield of cells. Glycerol acts as a source of carbohydrate. In contrast to glucose, no acetic acid is formed due to fermentation. Potassium phosphates are the buffering agents in the medium and prevent cell death due to a pH drop.

Cultural characteristics after 18-24 hours at 35°C.

Organisms (ATCC)	Growth
<i>Escherichia coli</i> C600 (23724)	+++
<i>Escherichia coli</i> HB101 (33694)	+++
<i>Escherichia coli</i> DH-1 (33849)	+++
<i>Escherichia coli</i> JM103 (39403)	+++
<i>Escherichia coli</i> JM107 (47014)	+++
<i>Escherichia coli</i> DH-5 (55868)	+++
<i>Bacillus subtilis</i> (6051)	+++

### References:

1. K.D. Tartoff, C.A. Hobbs, Improved Media for Growing Plasmid and Cosmid Clones, Bethesda Res. Lab. Focus 9, 12 (1987)
2. J. Sambrook, E.F. Fritsch, T. Maniatis, Molecular Cloning: A Laboratory Manual 2<sup>nd</sup> Ed., Cold Spring Harbor Labor. Press A2, (1989) Cold Spring Harbor