

## GROWTH MEDIA

### 5 - Trypan blue and Tetracycline medium (TB-T)

Components	Concentration (g/L)
Glucose (stock solution)	2
L-asparagine (stock solution)	1
NaHCO <sub>3</sub>	1
KH <sub>2</sub> PO <sub>4</sub>	0.5
MgSO <sub>4</sub> · 7H <sub>2</sub> O (stock solution)	0.1
Trypan blue (stock solution)	0.05
Tetracycline (stock solution)	0.02

#### PROCEDURE:

Prepare the following stock solutions in distilled H<sub>2</sub>O:

- Glucose 50% (store at room temperature)
- MgSO<sub>4</sub> · 7H<sub>2</sub>O 10g/L (store at room temperature)
- Trypan blue 0.1% (store at + 4 °C)
- L-asparagine 1.5% (store at + 4 °C)

Prepare the following stock solution in ethanol 50%:

- Tetracycline 12.5mg/ml (store in the dark at -20 °C)

Autoclave at 121 °C for 15 min glucose and MgSO<sub>4</sub> · 7H<sub>2</sub>O stock solutions and sterilize by filtration (pore size 0.22 µM) L-asparagine, tetracycline and trypan blue stock solutions.

Dissolve NaHCO<sub>3</sub> and KH<sub>2</sub>PO<sub>4</sub> in the appropriate volume of distilled H<sub>2</sub>O, taking into account the volume of other components, prepared as stock solutions, that must be added after sterilization. Adjust the pH to 5.5 with 10% phosphoric acid (4ml/L) and autoclave at 121 °C for 20 min. After sterilization, add glucose, MgSO<sub>4</sub> · 7H<sub>2</sub>O, trypan blue, L- asparagine and tetracycline.

To prepare solid medium, add agar (20g/L) before sterilization.