

## GROWTH MEDIA

### 8 - Nitrogen-free semisolid medium (BAz)

Components	Concentration (g/L)
Azelaic acid	2
K <sub>2</sub> HPO <sub>4</sub>	0.4
KH <sub>2</sub> PO <sub>4</sub>	0.4
MgSO <sub>4</sub> · 7H <sub>2</sub> O	0.2
CaCl <sub>2</sub> (stock solution)	0.02
Na <sub>2</sub> MoO <sub>4</sub> · H <sub>2</sub> O (stock solution)	0.002
FeCl <sub>3</sub> (stock solution)	0.01
Bromothymol blue (stock solution)	0.075
Agar	2.3

#### PROCEDURE:

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

- CaCl<sub>2</sub> 2%
- Na<sub>2</sub>MoO<sub>4</sub> · H<sub>2</sub>O 0.2%
- Bromothymol blue 5g/L (store at room temperature)
- FeCl<sub>3</sub> 1%

Autoclave at 121 °C for 20 min CaCl<sub>2</sub>, Na<sub>2</sub>MoO<sub>4</sub> · H<sub>2</sub>O and FeCl<sub>3</sub>.

Dissolve azelaic acid, K<sub>2</sub>HPO<sub>4</sub>, KH<sub>2</sub>PO<sub>4</sub>, MgSO<sub>4</sub> · 7H<sub>2</sub>O 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. Before sterilization, adjust the pH to 5.7 with KOH, then autoclave at 121 °C for 20 min. After sterilization, add CaCl<sub>2</sub>, Na<sub>2</sub>MoO<sub>4</sub> · H<sub>2</sub>O, FeCl<sub>3</sub> and filter-sterilized bromothymol blue stock solutions.