## ATCC Medium 2722: Supplemented Tryptic Soy Broth

Tryptic Soy Broth	30.0 g
Yeast Extract	
L-cysteine hydrochloride	0.5 g
Hemin Stock (see below)	1.0 mL
Vitamin K1 Stock* (5 mg/ml)	0.2 mL
DI Water	1000 mL

Autoclave at 121°C.

Wrap each bottle in aluminum foil or keep bottle in the dark as hemin and vitamin K are light sensitive and will be degraded.

Supplemented TSB can be pre-reduced and pre-warmed by incubating a bottle of fresh media in the anaerobic chamber overnight before using it. \*Menadione (vitamin K3) can be used at a final concentration of 1.0 mcg/ml instead of vitamin K1.

## **Hemin Stock Solution**

Hemin	0.5 g
K <sub>2</sub> HPO <sub>4</sub>	1.74 g
DI Water	100 mL

Boil the above ingredients in a large flask. Store in the dark.

Note: Hemin will have a final concentration of 5 mcg/mL. Vitamin K1 will have a final concentration of 1.0 mcg/mL

## Final Product Description



- Medium should be translucent and tan in color.
- > Recommended storage temperature: 2-8°C.