

**ATCC medium: 2222 Cell cultivation medium for parasites**

Dulbecco's Minimal Essential Medium (DMEM)\*..450.0 ml  
Fetal Bovine Serum (FBS)\*\*.....50.0 ml

Aseptically combine DMEM and cooled heat-inactivated serum.

\*ATCC Cat. #30-2002

\*\*ATCC Cat. #30-2021

ATCC-tested fetal bovine serum (FBS) is available as ATCC Cat. # 30-2020 (500 ml) and Cat. # 30-2021 (100 ml). ATCC-distributed FBS is NOT heat-inactivated.

Thawserum: Remove the serum from frozen storage and place it overnight in a refrigerator at 2C to 6C. Transfer the bottles to a 37C water bath for approximately 5 minutes. Agitate the bottles from time-to-time in order to mix the solutes that tend to concentrate at the bottom of the bottle. Do not keep the serum at 37C any longer than necessary to completely thaw it. Thawing serum in a bath above 40C without mixing may lead to the formation of a precipitate inside the bottle. We don't recommend thawing the serum at a high temperature. After the serum has warmed in the 37C water bath, continue with the heat-inactivation process.

Heat-inactivate the FBS: Pre-heat water bath to 56C. There must be sufficient water to immerse the bottle above the level of serum. Mix thawed serum by gentle inversion and place serum bottle in the 56C water bath. (The temperature of the water bath will drop.) When the temperature of the water bath reaches 56C again, continue to heat for a additional 30 minutes. Mix gently every five minutes to insure uniform heating. Remove serum from water bath and cool.

To avoid contamination, do not allow the level of the water in the bath to come in contact with the lip of the screw cap. It is best to leave one inch between the serum level in the bottle and the lip of the cap and to fill the water bath to a level just slightly above the level of the serum.