PROTOCOL FOR THE HANDLING OF HEAT INACTIVATED SERUM

THAWING SERUM

Remove the serum from the freezer and *thaw overnight in a refrigerator at 2-6 °C.* Complete thawing at room temperature. Agitate occasionally during thawing, if possible. Turbidity and flocculant material may appear upon thawing or prolonged storage. They are commonly seen in most serum products and do not cause reduced growth promotion. Thawing the serum at temperatures above ambient is not recommended.

HEAT INACTIVATION

Heat inactivation may reduce growth promotion properties of serum, especially if performed incorrectly. Sera with higher protein content, such as calf or horse sera, are more likely to form a precipitate when heat inactivated than is fetal bovine serum. *Prior to heat inactivation, thaw the serum at room temperature. The serum should be gently and continuously swirled during the entire heat inactivation procedure. When the serum temperature reaches 56 °C, timing for 30 minutes is begun. It is critical that neither the temperature nor the time limit be exceeded, as any serum can form a precipitate or gel, particularly if heated excessively.* Furthermore, improper heat inactivation can also diminish growth promotion properties. However, the presence of a moderate amount of precipitates following heat inactivation is not unusual and does not necessarily imply reduced growth promotion.

PRECIPITATES

Normal serum may exhibit a precipitate upon proper thawing or following heat inactivation. This does not interfere with growth promotion for most applications. This precipitate or cloudiness also does not mean the serum is non-sterile. However, if a precipitate is undesirable, the sterile undiluted serum may be filtered through a sterile 0.45 micron filter to remove objectionable matter. If the serum cannot be re-filtered to the 0.45 micron level aseptically then a 0.2 micron filtration step should be subsequently performed to assure sterility. However, since even a properly performed filtration can remove some growth promotion properties, it may be necessary to decide if a comparison test of filtered vs. non-filtered serum needs to be done. Alternatively, it may be desirable to accept precipitate in some lots of serum if growth promotion is a critical factor.

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