

SOP-P022

Preparation of Working Medium Endothelial RPMI

Objective: To prepare working medium for the growth and propagation of rat pulmonary endothelial cells

Procedure:

1. In a sterile environment such as a laminar flow hood, measure out 180 ml RPMI medium with sodium bicarbonate and without L-glutamine (Sigma #R-7880) into a sterile graduated cylinder.
2. Add the following to the cylinder:
 - 10 ml heat-inactivated fetal calf serum (refer to SOP#-P015 for Heat Inactivation of Bovine Sera)
 - 10 ml heat-inactivated newborn calf serum (refer to SOP above)
 - 2 ml 200 mM l-glutamine (Sigma #G-7513)
 - 2 ml penicillin (10,000U/ml)/streptomycin (10 mg/ml) solution (Sigma #P-0781)
3. Immediately sterilize the medium by filtration through a sterile filter with a porosity of 0.2 μ m into a sterile container such as an autoclaved medium bottle with screw cap.
4. Label the container with content information, date, and your initials.
5. Store the medium refrigerated at 0-5°C. Expiration date is approximately one month after date of preparation.

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Verified by: _____ **Date:** _____
Print Name Sign Name