Protocol 001 - Preparing RT Collagen Coated Flasks for Chordoma Cell Line Culture

Introduction

This protocol will cover coating T flasks with Rat Tail Collagen I(RT Col I) for general chordoma cell line culture.

Materials

- Sigma Rat Tail Collagen (Sigma, Cat# C3867-1VL) ***CHECK LOT NUMBER FOR CONCENTRATION***
- Sterile ddH_2O
- T flasks
- 1X PBS, pH 7.4 (Thermo Fisher, Gibco, Cat# 10010023)

Procedure

Cell Culture Flask Preparation - Thawing Procedure

1. ***CONCENTRATION IS LOT DEPENDENT. USE "SOURCE" ON VIAL AS LOT NUMBER. CONCENTRATION TO BE WRITTEN ON 15mL CONICAL ALIQUOTS*** Dilute 2.9mg/mL of Rat Tail Collagen I at a **1:58 ratio** in ddH_2O for a **final concentration of 50 ug/mL**

   RT Collagen may have solidified in 4C. If this is the case just warm the collagen to room temperature, in the dark, and wait for it to melt

2. For **5x T25** flasks dilute **224 uL of RT Col I** into **12.78 mL of sterile ddH_2O**.

   For **5x T75 flasks**, multiply this by 3x. (3*224uL of RT Col I and 3*12.78mL of Sterile ddH2O = **672 uL of RT Col I** and **38.34 mL of sterile ddH_2O**)

3. Add **2.5 mL of RT CoL I dilution to T25** flasks and **7.5 mL to T75 flasks**

4. Incubate at **37°C for 1 hour**

5. Aspirate Rat Tail Collagen I Dilution and thoroughly wash with equal volume of 1X PBS, pH 7.4. Repeat 2x

6. Immediately use flasks or store at **4°C for up to 1 month**