SERVA Electrophoresis

PRODUCT INFORMATION

Collagen CS Solution 0.5 % Cat. No. 47257

Product description:

General	Collagen is the major structural component of extracellular matrices found in connective tissues and internal organs, but is most prevalent in the dermis, tendons and bones. Type I collagen is a heterodimer composed of two α_1(I) chains and one α_2(I) chain that spontaneously forms a triple helix scaffold at neutral pH and 37 °C.
Application	• Coating of surfaces or use as a solid gel providing a substrate for improved attachment of thin layers of cells • Formation of an *in vivo*-like 3D collagen matrix attached to the bottom of culture dish or as a floating pad in/on the culture media for cell growth, cell migration assays, cell interaction • Biochemical or pathological studies of standard cells and stem cells
Composition	5 mg/ml acid soluble collagen (Type I) from bovine calf dermis in 0.01 M HCl
Storage	Store solution at +2 °C - +8 °C

Preparation of collagen gels for 2- and 3-dimensional cell growth

A. Additional required material	• 1 M NaOH, sterile • 1 M HEPES, sterile • 10x RPMI 1640 medium, sterile • Cell suspension (high density) in growth medium • Laminar air flow (LAF) unit, sterile air supply, sterile 50 ml beaker and sterile pipettes, sterile petri dish, ca. 50 – 55 mm diameter (ca. 2 ''), incubator A **without** CO_2, incubator B **with** CO_2, pH meter, pH probe, sterile 0.1 M HCl and/or sterile 0.1 M NaOH All solution should be used refrigerated at a temperature of + 4 °C to + 10 °C.
B. Preparation of neutralized collagen solution (aseptic, with sterile tubes in LAF unit)	1. Mix 0.7 ml of 1 M NaOH with 1.0 ml 1 M HEPES buffer (= **1.7 ml of solution A**). 2. Mix 2 ml of 10x RPMI 1640 with **1.7 ml of solution A** (= **3.7 ml of solution B**). 3. Mix 16 ml of Collagen CS solution 0.5 % with **3.7 ml of solution B**. 4. Mix thoroughly but gently and avoid trapping air bubbles. 5. The pH of the solution should ideally be at 7.2 – 7.8. If necessary adjust pH by adding a few drops of either sterile 0.1 M HCl or sterile 0.1 M NaOH. 6. Pipette ca. 7 ml of the neutralized collagen solution into a sterile petri dish with a diameter of ca. 50 – 55 mm diameter to cover completely the bottom to a depth of 2 – 3 mm.
C. Gelation by incubation of neutralized collagen solution	Incubate the neutralized collagen solution for min. 60 min or up to 16 h (overnight) at 37 °C to initiate and complete gelation. **Note: Gelation occurs more rapidly in the absence of CO_2.** **Before use collagen fibrils may alternatively be dried according to the following protocol:** • After gelation leave the dish uncovered in a stream of sterile air within the LAF unit overnight or until it is dry. • Rinse fibrillar collagen with sterile water to remove salts and rehydrate the dried film. • After rehydration the collagen film can be used immediately for cell culture. • If it is allowed to dry again as described before, the collagen film can be stored for future use.
D. Types of cell preparations	• **2-dimensional:** Prepare a neutralized collagen according section B and C. Disperse cells with medium on collagen gel surface after gelation and incubate. • **Sandwiched:** Prepare a neutralized collagen according section B and C. Disperse cells with a small amount of medium on collagen gel surface after gelation ("bottom gel"). Pour a new layer of neutralized collagen solution (B.1.-5.) gently on top of the cell layer ("top layer gel"). For gelation, incubate for min. 60 min or up to 16 h (overnight) at 37 °C. Continue incubation for cell growth. • **3-dimensional:** Prepare a neutralized collagen according section B.1.-5. and C. Prior to gelation, suspend cells in neutralized collagen solution by mixing 10 % of cell suspension in medium and 90 % neutralized collagen solution. For gelation, incubate this mixture for min. 60 min or up to 16 h (overnight) at 37 °C. Continue incubation for cell growth.

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