SERVA Electrophoresis

# PRODUCT INFORMATION

## Collagen CS Solution 0.5 % Cat. No. 47257

### Product description:

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| **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 $\alpha_1$(I) chains and one $\alpha_2$(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<br>• 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<br>• 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

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| **A. Additional required material** | • 1 M NaOH, sterile<br>• 1 M HEPES, sterile<br>• 10x RPMI 1640 medium, sterile<br>• Cell suspension (high density) in growth medium<br>• 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<br>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**).<br>2. Mix 2 ml of 10x RPMI 1640 with **1.7 ml of solution A** (= **3.7 ml of solution B**).<br>3. Mix 16 ml of Collagen CS solution 0.5 % with **3.7 ml of solution B**.<br>4. Mix thoroughly but gently and avoid trapping air bubbles.<br>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.<br>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.<br>**Note: Gelation occurs more rapidly in the absence of $CO_2$.**<br>**Before use collagen fibrils may alternatively be dried according to the following protocol:**<br>• After gelation leave the dish uncovered in a stream of sterile air within the LAF unit overnight or until it is dry.<br>• Rinse fibrillar collagen with sterile water to remove salts and rehydrate the dried film.<br>• After rehydration the collagen film can be used immediately for cell culture.<br>• 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.<br>• **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.<br>• **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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SERVA Electrophoresis GmbH • D-69115 Heidelberg • Carl-Benz-Str. 7
Tel.: +49(0)6221 / 138 40-0 • Fax• +49(0)6221 / 138 40-10 • E-Mail: info@serva.de http://www.serva.de