Preparation of Collagen Gel for Cell Culture
(for 2-dimensional and 3-dimensional cell growth)

A. Materials

1. Collagen G (0.4% collagen solution) or Collagen G1 (0.5% collagen solution), stored at +2° to +10°C, MATRIX BioScience GmbH
2. 1M sodium hydroxide (NaOH), sterile, SIGMA-ALDRICH, S 2770-100 mL
3. 1M HEPES buffer, sterile
4. 10x concentrate of RPMI 1640 medium, sterile, SIGMA-ALDRICH
5. Sterile 50 mL beaker
6. LAF-unit, sterile air supply
7. Sterile pipettes
8. Sterile Petri dish, approx. 50 – 55 mm (approx. 2 “)
9. Incubator A (without CO_2), Incubator B (with CO_2)
10. Miscellaneous: pH meter, pH probe, sterile 0.1M HCl and/or sterile 0.1M NaOH
11. Cell suspension (high density) in growth medium

All solutions should be used refrigerated at +2°C to +10°C.

B. Preparation of neutralized collagen solution (aseptic, with sterile tubes under LAF-unit)

1. Mix 0.8 mL of 1M NaOH with 1.0 mL of 1M HEPES buffer (= 1.8 mL of solution A)
2. Mix 2 mL of 10x RPMI 1640 with 1.8 mL of solution A (= 3.8 mL of solution B)
3. Mix 16 mL of 0.4-0.5 % collagen solution with 3.8 mL of solution B.
   Mix gentle and thorough, avoid trapping of air bubbles.
   The pH of the solution should ideally be at pH 7.8 - 8.0.
   If necessary adjust the pH by the addition of a few drops of sterile 0.1M HCl or 0.1M NaOH.
4. Pipette (or pour) the neutralized collagen solution into a sterile Petri dish.
   Example: Pipette approx. 7 mL of the neutralized collagen solution into a sterile Petri dish with a diameter of 50-55 mm (approx. 2 inches) to completely cover the bottom of the Petri dish to a depth of 2-3 mm.

C. Gelation / fibrillogenesis by incubation of neutralized collagen solution

1. Incubate the neutralized collagen solution for a minimum of approx. 60 minutes or up to 16 hours (overnight) at 37°C to initiate and to complete collagen fibrillogenesis / gelation.

NOTE: Gelation occurs more rapidly in the absence of CO_2.
NOTE: Before use, collagen fibrils may alternatively be dried in the following manner:

a) After gelation leave dish uncovered in a stream of sterile air under the LAF-unit overnight or until dry.
b) Rinse fibrillar collagen film with sterile water in order to remove salts and to rehydrate the film.
c) Collagen film can be used immediately for cell culture or allowed to dry again with sterile air and be stored for future use.

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D. Types of cell preparations:

2-dimensional : Prepare a neutralized collagen gel according to B.1. to B.4. and C.1.
Disperse cells with medium on the surface of the collagen gel after gelation and incubate.

sandwiched : Prepare a neutralized collagen gel according to B.1. to B.4. and C.1.
Disperse cells with a small amount of medium on collagen gel after gelation (on the surface of the “bottom gel”).
Pour a new layer of neutralized collagen solution (see B.1. to B.3) gently on top of the cell layer (for “top layer gel” formation).
Incubate the neutralized collagen solution for a minimum of approx. 60 minutes or up to 16 hours (overnight) at 37°C to initiate and to complete collagen fibrillogenesis / gelation of the “top collagen layer”.
Continue incubation for cell growth of sandwiched cell layer.

3-dimensional : Prepare a neutralized collagen solution according to B.1. to B.3.
Prior to gelation, suspend cells in neutralized collagen solution by mixing 10 % of a cell suspension in medium with 90% of the neutralized collagen solution.
Incubate the mix of neutralized collagen solution and cells for a minimum of approx. 60 minutes or up to 16 hours (overnight) at 37°C to initiate and to complete collagen fibrillogenesis / gelation.
Continue incubation for cell growth.

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