



MP Biomedicals, LLC

29525 Fountain Parkway  
Solon, Ohio 44139

Telephone: 440/337-1200  
Toll Free: 800/854-0530  
Fax: 440/337-1180  
mailto: [biotech@mpbio.com](mailto:biotech@mpbio.com)  
web: <http://www.mpbio.com>

## TECHNICAL INFORMATION

Catalog Number: 150703, 193492

### Collagen Solutions

Collagen Solutions are highly purified (predominantly Type I) solutions especially prepared for Tissue Culture applications. Collagen Solutions may be used to prepare a collagen coating on plastic or glass culture dishes, or to prepare actual collagen gels for culture work.

Since collagen plays an important role in cell culture by maintaining the physical function of cell support or attachment, the Collagen Solutions provide a convenient, efficient way to put a uniform collagen coating in culture dishes. The actual collagen "fiber" that is formed has been shown to be very effective, for example, in maintaining the albumin-producing function of hepatocytes cultured on it.

Collagen Solutions may also be used to form actual culture gels, and human fibroblasts cultured in these gels are able to condense the collagen to a corium-like structure by cell contraction.

Three (3) different Collagen Solutions are presently available:

| Catalog Number | Description                                                                              | Sizes Available       |
|----------------|------------------------------------------------------------------------------------------|-----------------------|
| 150703         | Collagen Solution, acid solubilized, 3 mg/ml                                             | 1 ml<br>5 ml<br>10 ml |
| 151458         | Collagen Solution, enzyme solubilized, 3 mg/ml                                           | 150 mg<br>300 mg      |
| 193492         | Collagen Solution, acid solubilized, containing 0.3% collagen buffered with 0.1% acetate | 20 ml                 |

Collagen Solutions are supplied ready-to-use.

### Selected Methods for the Use of Collagen in Cell Culture

#### I. Preparation of Collagen Gels

##### A. Materials

- Collagen, chilled to a temperature of 4-6°C.
- Sterile 10X phosphate-buffered saline solution (0.2 M Na<sub>2</sub>HPO<sub>4</sub>, 1.3 M NaCl, pH = 7.4).
- 0.1 M HCl
- 0.1 M NaOH
- Phenol Red or pH paper

##### B. Preparation of neutralized, isotonic collagen solutions

- Mix 8 ml of chilled collagen with 1 ml of 0.1 M NaOH and 1 ml of 10X phosphate buffered saline solution. (Alternatively one can use a 10X solution of buffered cell culture media.)
- Adjust the pH of the solution to 7.4 ± 0.2 by the addition of a few drops of 0.1 M HCl or 0.1 M NaOH. The pH of the solution can be monitored by pH paper or by the use of a pH indicator (dye) such as phenol red. Phenol red can be added to the phosphate buffered saline solution at a concentration of 0.005 mg/ml.
- The neutralized, isotonic collagen solution can be stored at 4-6°C for several hours prior to gelation.

##### C. Gelation of neutralized, isotonic collagen solutions.

- Collagen gelation (fibrillogenesis) can be initiated by warming the neutralized collagen solution to 37°C. Allow 10-20 minutes for gelation to occur.
- Cells can be dispersed on collagen gels, sandwiched between collagen gels or suspended in collagen gels by mixing them with the neutralized collagen solution prior to gelation.

## II. Preparation of Fibrillar Collagen Films for Covering Cell Culture Surfaces

- A. Materials are the same as described above for preparing collagen gels.
- B. Preparation of collagen films.

- Prepare neutralized, isotonic collagen solution as described above.
- Cover surface with this solution to a depth 1-2 mm (1-2 ml for a 35 mm cell culture dish).
- Incubate for 10-20 minutes at 37°C to promote gelation.
- Leave dish uncovered in laminar flow hood overnight or until dry.
- Rinse film with sterile water in order to remove salts and rehydrate film.
- Film can be used immediately for cell culture or allowed to dry again and be stored for future use.

## III. Preparation of Monomeric Coating for Covering Cell Culture Surfaces

### A. Materials

- Collagen solution
- Sterile air supply (Optional).

### B. Preparation of collagen films

- Place a thin layer of collagen inside the dish or well to be coated.
- Take to dryness in a stream of sterile air or alternatively leave the dish uncovered in a laminar flow hood overnight to allow for normal evaporation.
- Rinse dish with sterile buffered isotonic saline solution or media to remove residual acid and rehydrate collagen prior to use.
- Collagen coatings prepared in this manner are nonfibrillar in nature and thus can be distinguished from the fibrillar collagen preparations described above.

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