

### ★ Storage

Store at 4 °C.  
The product ships on frozen gel packs with storage at 2-10 °C recommended.  
Do not freeze.  
The expiration date is printed on the product label.  
The expiration date is applicable when product is handled and stored as directed.

### ★ Contents

- Product Manual
- Ceracol, Collagen Type I Solution in 0.025M Acetic acid, 3mg/ml, pH 2.0

ALL PRODUCTS SOLD BY GenDEPOT ARE INTENDED FOR RESEARCH USE ONLY UNLESS OTHERWISE INDICATED. THIS PRODUCT IS NOT INTENDED FOR DIAGNOSTIC OR DRUG PURPOSE

### ★ Shipping Condition

The product ships on frozen gel packs with storage at 2-10 °C recommended.

### ★ Introduction

Ceracol Collagen Type I solution, is approximately 3mg/ml, pH 2, available in 100ml volume, and produced by aseptic processing. Ceracol is about 97% Type I collagen with the remainder being comprised of Type III collagen. Ceracol contains a high monomer content as judged by gel permeation chromatography.

Type I collagen is a major structural component of skin, bone, tendon, and other fibrous connective tissues, and differs from other collagens by its low lysine hydrox-ylation and low carbohydrate composition. Although a number of types of collagen have been identified, all are composed of molecules containing three polypeptide chains arranged in a triple helical conformation. Slight differences in the primary structure (amino acid sequence) establish differences between the types. This product is suitable for the preparation of collagen gels for the culture of human hematopoietic progenitor cells. This product can also be used to prepare collagen films (collagen coating) on cell culture surfaces.

The rate of gel formation, gel consistency, and gel clarity may vary between lots. Agitation of the gel during formation, exposure to ultraviolet light and temperature extremes can influence the integrity of the gel.

### ★ Concentration

The concentration of this product is approximately 3.0mg/ml.

### ★ Protocol

Coating Procedure for fibroblast cell lines

1. Place an appropriate amount of Ceracol to the culture surface.

Note: 0.6 - 1ml per 35mm dish (0.06-0.1 ml per cm<sup>2</sup>)

Determine the optimal coating conditions for your culture system.

Note: Less than 0.5ml of solution per 35mm dish cannot be spread over the full surface of the dish because the collagen solution is high in viscosity.

When reducing the amount of collagen per culture dish, dilute the collagen solution with 0.025M Acetic acid solution.

2. In a sterile hood, open the cap of the culture dishes and air-dry for 30-60 minutes or until the surface is completely dry.
3. Rinse the culture dish with sterile PBS 2-3 times so that it neutralizes.
4. Dried coated dishes can be sterilized 30-60 minutes by exposure to UV light in a sterile tissue culture hood.
5. Rinse the dish once with sterile PBS or medium.
6. Coated surface are ready for use. Pour the cell suspension into the dishes and culture as usual.

**Note:** The dishes can be used immediately or stored under sterile conditions for up to two weeks at 4°C.

### Coating Procedure for human mammary epithelial cells

1. Transfer desired volume of collagen solution from the bottle to a dilution vessel as required. Dilute to desired concentration using sterile 0.025M Acetic acid solution. A typical working concentration may range from 10 to 100 ug/ml. (1:300 - 1:30)  
Note: Determine the optimal concentration for your culture system.
2. Place an appropriate amount of Ceracol to the culture system.

**Note:** 0.6-1ml per 35mm dish (0.06-0.1 ml per cm<sup>2</sup>)

Determine the optimal coating conditions for your culture system.

**Note:** Less than 0.5ml of solution per 35mm dish cannot be spread over the full surface of the dish because the collagen solution is high in viscosity.

3. In a sterile hood, open the cap of the culture dishes and air-dry for 30-60 minutes or until the surface is completely dry.
4. Rinse the culture dish with sterile PBS 2-3 times so that it neutralizes.
5. Dried coated dishes can be sterilized 30-60 minutes by exposure to UV light in a sterile tissue culture hood.
6. Rinse the dish once with sterile PBS or medium.
7. Coated surface are ready to use. Pour the cell suspension into the dishes and culture as usual.

**Note:** The dishes can be used immediately or stored under sterile conditions for up to two weeks at 4°C.

### 3-D Gel Preparation Procedure

1. Prepare collagen gel medium. Mix each reagent in chilled condition. If serum is required, add the serum. Mix the solution by pipetting until the collagen solution is completely mixed up.

< for 10ml collagen gel medium >

Chilled Medium or PBS (5X concentration) 2ml

Chilled Ceracol 8ml

2. After mixing, keep the solution on ice. Adjust pH of mixture to 7.2-7.6 using sterile 0.1M NaOH (200-250ul NaOH for 10ml mixture). The pH of the collagen gel solution should be neutral, which is indicated by the pink/red color of phenol red in the 5X medium.

### 3-D Culture: WHEN CULTURING CELLS ON THE TOP OF COLLAGEN GEL

1. Pipette a proper size of a chilled collagen gel solution onto a tissue culture plate of dish.

**Note:** Suggested Collagen Gel Amounts Culture

2. Immediately transfer to 37 °C incubator for 60 minutes to initiate polymerization of the collagen. The polymerized gel will look cloudy.
3. After formation of the collagen gel, seed desired cells (0.1 to 2.0 X10<sup>6</sup> cells/ml) onto the collagen gel.
4. Overlay polymerized collagen gel with culture media.
5. Incubate cells overnight or several days at 37°C with CO<sub>2</sub>. Change medium daily.
6. Cells can be visualized using phase contrast microscopy and can be directly fixed and stained within the collagen.

### ★ Related GenDEPOT Products

Product Name	Cat No
DMEM, High Glucose, without Sod Bicarbonate	CM001
DMEM, High Glucose, with Sod Bicarbonate	CM002
MEM with Earle's salt	CM041
RPMI-1640 with HEPES	CM058
RPMI-1640	CM059
Trypsin-EDTA(1X)	CA014
Trypsin-EDTA(10X)	CA015
Scraptase, Non-Animal Origin Dissociation Solution(1X)	CA110
Dispase Solution, 1mg/ml	CA092
Collagenase/Hyaluronidase(10X) in DMEM	CA094
Antibiotics-Antimycotics(100X)	CA002
Penicillin-Streptomycin(100X)	CA005
Dulbecco's Phosphate-Buffered Saline(DPBS)(1X)	CA008
Cellmaxin, 10mg/ml	C3314
Cellmaxin Plus, 10.5mg/ml	C3319
Ceracryo, Xeno-Free Cell Freezing Mediu	C0667
Water, 0.1um Filtered, Cell Culture Tested	W0900