Rat tail Tendon Collagen Type I

Cat: C8062
Size: 10mg, 5mg/mL, Dissolved in 0.006mol/L HAc, sterile.
Storage: Store at 4℃, do not freeze, valid for 1 year.

Introduction:

Rat tail tendon collagen type I was prepared by Birkedal-Hansen method through acetic acid extraction, sodium chloride precipitation and disodium hydrogen phosphate precipitation. Our company's rat tail collagen can be used in coated cell culture vessels to culture some cells that are not easy to stick to the wall in ordinary cell culture vessels. It can also be used to prepare three-dimensional glue to simulate the real growth environment, so that cells grow in a three-dimensional environment.

1. Adhesion and growth of PC-12 cells were examined in cell culture dishes coated with Solarbio rat collagen type I.
2. When the concentration was above 1mg/mL and the pH was about 7, the three-dimensional gel with certain strength could be formed. It was found that NIH-3T3 cells grew normally in the three-dimensional gel and PC-12 cells grew normally on the surface of the three-dimensional gel.

Protocols:

1. The recommended concentration for the surface envelope of cell culture vessels is 1-5μg/cm^2
Taking a coating concentration of 2μg/cm^2 as an example, collagen was diluted to 0.012mg/mL with sterile 0.006mol/L(0.36g/L) acetic acid.
Add to the appropriate culture vessel according to the volume in the table below

	Surface area(cm^2, per well or dish)	Add 0.012mg/mL collagen volume (μL)
96-well cell culture plate	0.3	50
24-well cell culture plate	1.9	300
12-well cell culture plate	3.8	600
6-well cell culture plate	9.5	1580
35mm cell culture dish	8	1330
60mm cell culture dish	21	3500
100mm cell culture dish	55	9170

Make sure the collagen solution covers the surface of the utensil and leave the lid on a super clean table overnight to dry. It can also be left at room temperature for 1h, Wash with PBS 3-4 times and then use directly. The well-wrapped utensils can be stored for at least 3 months at 4-25℃.

2. Preparation of three-dimensional collagen

Rat tail collagen type I can form a three-dimensional gel with certain strength when the concentration is above 1mg/mL and pH7 is about, so it is recommended to form a gel concentration of 1-2mg/mL. Collagen is dissolved in 0.006mol/L acetic acid, and 0.06× volume of 0.1mol/L NaOH is added to neutralize it during the gelation process.

Solution required(all sterile, pre-cooled): 10×PBS(can contain 10 mg/L phenol red for pH indication) or 10× culture solution, 0.1mol/L NaOH, 0.1mol/L acetic acid(usually not used), double steaming water.

A. Preparation of cell-free 3D collagen(as an example of preparing 1mL, 1mg/mL 3D gel for reference only): 200μL rat tail collagen Type I(5mg/mL) is added to a centrifuge tube placed in an ice bath with 690μL H_2O. Then add to 12μL 0.1mol/L NaOH(if reversed adding 12μL 0.1mol/L NaOH to the collagen solution will result in local collagen coagulation due to the failure of NaOH to mix quickly), mix immediately. Add 100μL of 10×PBS or 10×culture solution, mix and immediately add to the culture vessel(note that the pH after mixing should be about 7, if the PBS or culture solution is not added with phenol red, it is necessary to use a pH test strip for the first time). Place the culture vessel at room temperature(about 25 degrees) for 20min until the glue sets, then transfer it to the incubator. If 10×PBS is used in the preparation, it is necessary to add the appropriate volume of cell culture solution to pre-balance before use.

B. Preparation of three-dimensional collagen containing cells(as an example of preparing 1mL, 1mg/mL three-dimensional gel, for reference only): Prepare the cells suspended in a culture solution and place them in an ice bath. Add 200μL rat tail collagen Type I(5mg/mL) to 12μL 0.1mol/L NaOH(if reversed adding 12μL 0.1mol/L NaOH to the collagen solution will result in local collagen coagulation due to the failure of NaOH to mix quickly), mix immediately. Add 23μL of 10×PBS or 10× culture solution and mix well (note that the pH after mixing should be about 7, if no phenol red is added to PBS or culture solution, a pH test paper should be used for the first time). Add 760μL of cell suspension, mix well and immediately add to a culture dish. Leave the culture dish at room temperature for 20 minutes until the glue solidified, add the appropriate volume of cell culture solution, and transfer to the incubator for culture.

Note:

Rat tail collagen type I can quickly form into gel when pH is neutral at room temperature, and should be kept as low as possible during operation.