

Rat Type I Collagen-based Hydrogel

Order Information

Product Name:	Rat Type I Collagen-based Hydrogel
Catalogue Number:	cAP-07
Concentration:	1x Hydrogel (Ready to use)
Size:	50.0ml (For minimum of 50 assays using 24-well plate)
Storage:	< -20°C

General Information

Collagen based Hydrogel is a biocompatible and injectable complex of Type I Collagen biopolymers that can accelerate the pace of biomedical and cell/tissue engineering applications. Collagen-based Hydrogel contains high quality, sterile Type I rat tail collagen, which has been specially formulated for ease of gel formation.

Product Specifications

Source:	Rat Tails
Celsius Shelf life:	12 months
Storage:	< -20°C
Purity:	> 95% SDS PAGE
Concentration:	1 x Collagen based hydrogel (Ready to use)
Product pH:	7.2-7.4
Sterility:	Pass
Endotoxin Level :	< =1EU/ml
Cell Culture Testing:	Pass

Sterility Testing: This product has been tested after 14 days after incubation in a 37°C CO2 incubator. It is free of bacterial and fungal contamination. Product has shown to be negative with respect to mycoplasma contamination by Real-Time PCR.

Application: Endothelial cell tube formation (sandwich assay):

Material and reagents needed:

- A flask (T25) of endothelial cells
- 1x Hydrogel (cAP-07, ~1.0ml for one well when using 24-well plate)
- Endo-Growth media (cAP-02)
- Endo-Basal media (cAP-03)
- 24-well cell culture plate

Protocol (Using 24-well cell culture plate as an example):

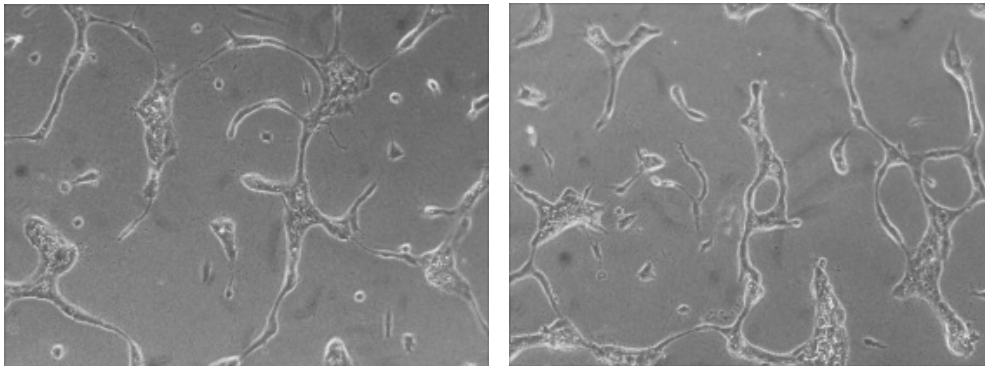
- 1) Thaw 1x Hydrogel at 2-4°C (on ice in a refrigerator) one night before the day of experiment;
- 2) Keep both 1x Hydrogel and culture media on ice before experiment;
- 3) Gently add 0.5ml ice-cold 1x Hydrogel in one well of 24-well plate (avoid generating air bubbles) and allow Hydrogel to be solidified by leaving the plate in a 37°C CO2 incubator for a minimum of 15 minutes;
- 4) While waiting, prepare endothelial cell suspension (2-4 x10⁵ cells/ml: dissociation of cells from

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- T25 flask with trypsin-EDTA and then re-suspend endothelial cells in Endo-Growth media;
- 5) Gently add 0.5ml ($1-2 \times 10^5$ cells/well) of ice-cold cell suspension in one well of 24-well plate and allow endothelial cells to be attached to bottom layer of Hydrogel by culturing endothelial cells in a 37°C CO2 incubator;
 - 6) When endothelial cells reach 95% confluent, gently but thoroughly aspirate off the Endo-Growth media from each well before adding 0.3-0.5ml ice cold 1x Hydrogel to form top layer of Hydrogel (avoid generating air bubbles);
 - 7) 1 hour after top layer of Hydrogel is solidified in a 37°C CO2 incubator, 0.5ml of Endo-basal media or Endo-basal media containing variable concentration of Fetal bovine serum (FBS) or experimental reagents is added gently on top layer of Hydrogel, according to user's experimental goal;
 - 8) Endothelial tubes formation can normally be observed after overnight incubation of the cells in a 37°C CO2 incubator.

Suggested volume of Hydrogel used for variable culture wares:

Culture wares	Area (cm ²)	Hydrogel Volume (ml)
96 well	0.143	0.1
24 well	0.33	0.5
12 well	1.12	1.0
6 well	4.67	2



Human microvascular endothelial tube formation (Sandwich) assay in 24-well plate using Collagen-based Hydrogel (cAP-07): Two representative images at 24hours after Endo-Basal media was added on top layer of Hydrogel.

This product is for R&D use only (not for human diagnostic and treatment in any forms)