

PRODUCT SPECIFICATION SHEET**RAT TAIL COLLAGEN High Concentration (HC), TYPE 1**

Collagen I is found in most tissues and organs, but is most plentiful in dermis, tendon and bones. Type I molecule is a heterotrimer [$\alpha_1(I)_2 \alpha_2(I)$] of 300 nm length being composed of two $\alpha_1(I)$ chains and one $\alpha_2(I)$ chain.¹ Collagen binding integrin receptors are $\alpha_1 \text{Beta}_1$, $\alpha_2 \text{Beta}_1$, and $\alpha_3 \text{Beta}_1$.² When used as a gel, collagen facilitates successful adaptation *in vitro* culture and enhances expression of cell-specific morphology and function. The Collagen HC is used in applications where a sturdy gel provides maximal support to maintain the three dimensional structure³⁻⁵.

CATALOG NUMBER: 354249 LOT NUMBER: _____

SOURCE: Rat tail tendon

QUANTITY: 100 milligrams protein (measured by pyrochemiluminescence)

CONCENTRATION: _____ mg/ml

FORMULATION: 0.02N Acetic Acid

USE: Rat Tail Collagen HC is used as a three dimensional gel. Please see reverse for gelling procedures. Use these as guidelines only - we recommend that each investigator empirically determine the optimal conditions for their unique applications.

QUALITY CONTROL: _____ by SDS PAGE

This product has been tested for its ability to promote the attachment and spreading of HT 1080 Human Fibrosarcoma cells.

Rat Tail Collagen HC, Type 1 is a membrane-filtered (0.2 micron) preparation. Tested and found negative for the presence of bacteria, fungi and mycoplasma.

STABILITY: Stable for at minimum of 3 months from day of shipment when stored at 2-8°C.

On release this product has been successfully gelled over a wide range of dilutions and will form a gel up to a concentration of 0.3mg/ml. Further dilution may decrease the rigidity of the gel as will the time from manufacture.

REFERENCES:

1. Linsenmayer, T.F., Collagen, in Cell Biology of Extracellular Matrix (ed., E.D. Hay) pp 5-37, Plenum Press, NY (1991).
2. Bosco, M., et.al., J. Cell Biol., **120**:537 (1993).
3. Gautreau, A. et.al., PNAS,96: 7300 (1999).
4. Abir, R. et.al., Hum Reprod, 14: 299 (1999).
5. Abir R., et.al., Fertil Steril, 75:141 (2001).

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Gelling Procedure- Rat Tail Collagen HC will gel when its pH is brought to alkalinity using the procedure below. Please use this as a guideline for determining the optimum concentration for your application.

- 1) Prepare ammonia vapor chamber by taping a sterile 2 inch gauze sponge to the inside lid of a 150mm petri dish. Saturate the gauze with ammonium hydroxide. Place lid on 150 mm dish and set aside.
- 2) Place an even coating of collagen on surface to be coated. Thickness may be varied as desired. 50 to 100 ul of collagen is sufficient to coat a 22mm coverslip. For dishes of 100mm diameter, add approximately 6.0 ml per dish; for 60 mm dishes add approximately 2.3 ml, and for 35 mm dishes add approximately 1.0 ml.
- 3) Transfer coated coverslips or dishes with lids off to ammonia vapor chamber and expose for three minutes.
- 4) Soak coated coverslip or dishes in sterile dH₂O for 30 minutes (5 ml for 35 mm dishes, 10 ml for 60 mm dishes, etc.). Aspirate and replace with 0.5 to 1 ml of sterile dH₂O and let sit overnight lidded in a laminar flow hood.
- 5) Aspirate the dH₂O and replace with serum supplemented balanced salt solution and store at 2-8°C.

Alternate Gelation Procedure for Rat Tail Collagen HC

- 1.0 Place on ice the following:
 - 1.1 Rat Tail Collagen, P/N354249
 - 1.2 Sterile 10X phosphate buffered saline (10X PBS)
 - 1.3 Sterile dH₂O
 - 1.4 Sterile 1N NaOH
- 2.0 Determine the final volume of collagen solution to be used and the desired final collagen concentration.
- 3.0 Place on ice a sterile tube of sufficient capacity to contain the final volume of collagen.
- 4.0 Perform the following steps using aseptic technique in a Class 100 Hood.
 - 4.1 Add to the tube the following volume of 10X PBS:
$$\frac{\text{Final Volume}}{10} = \text{ml 10X PBS}$$
 - 4.2 Calculate the volume of collagen to be used (do not add to the tube until step 4.6):
$$\frac{\text{Final volume} \times \text{Final collagen concentration in mg/ml}}{\text{Concentration in bottle (see spec sheet)}} = \text{volume collagen to be added}$$
 - 4.3 Add to the 10X PBS the following volume of sterile ice cold 1N NaOH:
(volume collagen to be added) x 0.023 ml = volume 1N NaOH
 - 4.4 Add to the 10X PBS/1N NaOH the following volume of sterile ice cold dH₂O:
(Final volume) - (Volume collagen) - Volume 10X PBS) - (Volume 1N NaOH) = volume dH₂O to add
 - 4.5 Mix the contents of tube and hold in ice.
 - 4.6 Add the calculated volume of collagen and mix - leave on ice until ready for use.
- 5.0 The collagen solution can be used immediately or held on ice for 2-3 hours.
- 6.0 When ready to use, aseptically deliver the solution into the cell culture device and allow to gel at 37°C for 30 minutes.

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.