

Certificate of Analysis

CORNING® COLLAGEN I, HUMAN

Collagen I is found in most tissues and organs, but is most plentiful in dermis, tendon and bones. The 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.^{1,2} Collagen binding integrin receptors are $\alpha_1 \text{Beta}_1$, $\alpha_2 \text{Beta}_1$, and $\alpha_3 \text{Beta}_1$.³ When used as a gel, collagen facilitates successful adaptation in vitro culture and enhances expression of cell-specific morphology and function. Collagen may also be used in a thin layer to promote attachment. Applications include the study of tumor cell invasion and migration,^{4,5} fibrillogenesis studies,⁶ culture and/or differentiation of monocytes macrophages and/or macrophages,⁷ and autoradiographic studies of granulocytes and macrophages.⁸ Collagen I is also used in the maintenance of hepatocyte function, state of differentiation and elevated levels of liver cell gene transcription.^{9,10} Collagen gels will maintain the differentiated state of cultured avian skeletal myotubes,¹¹ and can be used to study secretory epithelium¹² and growth patterns of normal and neoplastic mammary cells.^{13,14}

CATALOG NUMBER: 354243

LOT NUMBER: 4272009

SOURCE: Human placenta

NOTE: Any of the human source material used in the manufacturing of this material was tested and found nonreactive for hepatitis B surface antigen (HBsAG), for antibodies to the following viruses, hepatitis C virus (anti-HCV), human immunodeficiency virus-1 (anti-HIV-1), and human immunodeficiency virus-2 (anti-HIV-2). Regardless of the test data this product should be handled observing the same Universal Safety Precautions employed when handling any potentially infectious material.

QUANTITY: 0.25 milligrams

CONCENTRATION: 3.58 mg/mL (measured by Chemiluminescence)

FORMULATION: 2 mM Hydrochloric acid (HCl)

PURITY: $\geq 90\%$ by SDS-PAGE

USE: Corning Collagen I, human, is generally used as a thin coating, but may be used as a gel if desired. Please see reverse for coating procedures. These are guidelines only - we recommend that each laboratory empirically determine the optimal conditions for their unique applications. If material is not to be used all at once, dispense into appropriate aliquots and store at -20°C .

QUALITY CONTROL: Corning Collagen I, human, is a membrane filtered ($0.2 \mu\text{m}$) preparation. Tested and found negative for the presence of bacteria, fungi and mycoplasma.

STORAGE: Stable when stored at -20°C . Avoid multiple freeze-thaws. Do not store in frost-free freezer. **KEEP FROZEN.**

EXPIRATION DATE: November 30, 2016

- REFERENCES:
1. Kuhn, K. The Classical Collagens: Type I, II and III in Structure and Function of Collagen Types (R. Mayne and R. E. Burgeson, eds.) pp 1-42, Academic Press, NY (1987).
 2. Linsenmayer, TF. Collagen, in Cell Biology of Extracellular Matrix (ed., E.D. Hay) pp 5-37, Plenum Press, NY (1991).

3. Chan, B.M., and Hemler, M.E., *J. Cell Biol.*, **120**:537 (1993).
4. De Wever, O., et.al., *Int. J. Dev. Biol.*, **54**:887 (2010).
5. Baker, E.L., et.al., *PLoS One.*, **6**:e20355 (2011).
6. Gobeaux, F., et.al., *J. Mol. Biol.*, **376**:1509 (2008).
7. Wesley, R.B. II., et.al., *Arterioscler. Thromb. Vasc. Biol.*, **18**:432 (1998).
8. Izumi, T., et.al., *J. Cell. Physiol.*, **126**:155 (1986).
9. Sidhu, J.S., et.al., *Arch. Biochem. Biophys.*, **301**:103 (1993).
10. Gómez-Lechón, M.J., *J. Cell Physiol.*, **177**:553 (1998).
11. Vandeburgh, H.H., et.al., *In Vitro Cell Dev. Biol.*, **24**:166 (1988).
12. Hall, H.G., and Bissell, M.J., *Exp. Cell Res.*, **162**:379 (1986).
13. Azzam, H.S., and Thompson, E.W., *Cancer Res.*, **52**:4540 (1992).
14. Streuli, C.H., et.al., *J. Cell. Biol.*, **120**:253 (1993).

Suggested Coating Procedures

Corning® Collagen I, human, has been shown to promote the attachment of HT-1080 (human fibrosarcoma) cells at concentrations as low as 0.1 µg/cm². The optimal concentration for cell attachment and culture may differ for different cell types, and experimentation may be required to determine the optimal conditions for your cell culture system.

Thin Coating

1. Add sufficient volume of Corning Collagen I, human, to provide desired coating concentration. We recommend using a coating concentration of 0.2-2.0 µg/cm² depending on the cell type. Be sure that the volume added to the dish is sufficient to cover the growth surface. If necessary, dilute the Corning Collagen I, human, stock with 2 mM HCl.
2. Once growth surface has been completely covered, incubate for 2 hours at room temperature; tilt dish at 45° angle and allow excess Corning Collagen I, human, to drain to the lowest point in dish.
3. Remove excess material with sterile Pasteur pipette.
4. Air dry plates by leaving lids ajar in a laminar flow tissue culture hood, or dry with a gentle stream of sterile gas.
5. Plates are now ready for use.

Gelling Procedure

1. Dilute Corning Collagen I, human, to desired concentration using 2 mM HCl.
2. Mix together nine parts Corning Collagen I, human, and one part 10X buffer or 10X media.
3. Add mixture to desired tissue culture vessel.
4. Incubate for 15 to 60 minutes at 37°C. Gel is ready for use, but must be handled carefully.

NOTE: For more details on Corning Collagen products and technical resources please visit support page at www.corning.com/lifesciences

Quality Assurance

Date