Instructions for Use

HumaDerm- *Human Skin Collagen Type I, Lyophilized*

For 2D-3D tissue engineering research, hydrogels and cell culture applications

SKU: SCL
Tissue Source: Human Skin

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Product Description

Type I Collagen is the most abundant type of collagen in the human body, as a major structural matrix protein in skin, and many other tissues (bone, tendon, and fibrous connective tissues) [1]. There are a number of types of collagen identified to date, and all are composed of molecules containing three polypeptide chains arranged in a triple helical conformation [2]. The types of collagen differ slightly in the primary amino acid sequence of their polypeptide chains. Type I collagen is a heterotrimer composed of one α_2(I) chain and two α_1(I) chains [3].

Various types of cell surface receptors present on diverse types of mammalian cells recognize the collagen triple helix structure and facilitate cell attachment to collagen materials, including films and scaffolds. The most characterized cell receptors are the integrins $\alpha_1\beta_1$ and $\alpha_2\beta_1$ [4]. Many cell types express both forms of integrin, including mesenchymal stem cells (MSCs), fibroblasts, endothelial cells, chondrocytes, osteoblasts, and lymphocytes[3, 5-7]. Smooth muscle cells interact with collagen via $\alpha_1\beta_1$, and epithelial cells attach via $\alpha_2\beta_1$ [8, 9]. Collagen type I is characterized by the presence of three regions on SDS-PAGE corresponding to molecular weights. Alpha region has a molecular weight of 100 KDa and consists of two α_1 chains and one α_2 chain. Beta region has a molecular weight of 200 KDa and consists of two α_1 chains fused together and α_1 α_2 chains fused together. Gamma region has a molecular weight of 300 KDa and consists of overlapping two α_1 and one α_2 chains**. HumaDerm** has all three regions and chains with very minimal fragmented proteins in between. **HumaDerm** is ideal for many applications including coating tissue culture surfaces to support cell attachment and growth or for making hydrogel.

Source Tissue

Type I collagen is isolated from human skin sourced strictly from American Association of Tissue Banks (AATB) accredited and FDA registered tissue banks and organ procurement organizations (OPOs). Humabiologics strives to meet research needs by providing high quality biomaterials obtained from tissue partners who comply with requirements for transplantable human tissues under 21 CFR 1271 of the Food and Drug Administration (FDA).

Precautions and Disclaimer

HumaDerm is obtained from human tissue that has been tested and found negative for minimum of HIV-1 and -2, hepatitis B, and hepatitis C, as well as other infectious agents. Please review the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices. ***HumaDerm is for research use only and is not intended for human use, diagnosis, screening, household, food or other uses.***

Storage/Reconstitution

HumaDerm should be stored at -20 °C upon receipt. The expiration date is 1 year from receipt of the bottle. Collagen is a stable protein if it is stored frozen while minimizing freeze-thaw cycles.

HumaDerm can be reconstituted in HCl or acetic acid. **HumaDerm** is soluble in 10-20 mM HCl with a final pH of 1.9-2.1. Reconstitution may take 3 hours at ambient temperature with vigorous shaking/vortexing or overnight at 4ºC. Solution can be briefly centrifuged to remove bubbles. Do not let the temperature exceed 30°C during reconstitution. After reconstitution, solution should be used immediately or aliquoted and frozen at -20 °C.

Collagen-Coated Tissue Cultureware

Note: The following are general recommendations. Researcher should optimize parameters based on their specific applications

1. Reconstitute **HumaDerm** to 0.1 mg/ml in 10-20 mM HCl with a pH of 1.9-2.1. It is recommended to use ultrapure sterile water.
   NOTE: Different concentrations may need to be tested to determine the optimal concentration for each culture system, typical coating concentrations range between 10 – 100 µg/ml.
2. Mix by shaking or vortexing until the solution is homogeneous.
3. Add the desired volume of diluted collagen solution to surface of cultureware and spread the solution to cover the entire surface. Typically, plates are coated at 125 µl/cm^2.
4. Store aliquots at -20 °C if not used immediately.
5. Cover the coated cultureware and incubate at 37 °C for at least 2 hours.
6. Aspirate excess solution on coated surface. Avoid scratching the coated surface.
7. Leave coated culture surface to dry overnight in a cell culture hood with the lid off. UV light exposure for 30 minutes may be used to sterilize the wells.
8. Rinse the coated culture surface with sterile PBS or culture medium to remove residual acid.
9. Use coated cultureware immediately or keep sterile and store at 2-10 °C.

Making 3D Collagen Gels

Note: All procedures should be performed in a cold room or on ice. All reagents should be cooled for 30 minutes prior to making the hydrogel

1. Reconstitute **HumaDerm** in 10-20 mM HCl with a pH of 1.9-2.1. It is recommended to use ultrapure sterile water.
   *NOTE: Concentrations will need to be tested to determine the optimal gel concentration for each application. **HumaDerm** has been tested and found to form a gel at ≥ 0.5 mg/ml final concentration.*
2. Prepare 10x culture medium or 10x PBS.
3. Combine 9 volumes of chilled collagen solution with 1 volume of chilled 10x concentrated medium or PBS.
4. Gently swirl or pipette the mixture repeatedly.
   NOTE: To prevent premature gelation, keep combined solution between 2-10 °C
5. Adjust the pH to 7.0-8.0, preferably around 7.4. Concentration and pH will affect the speed and strength of the gel.
6. Incubate at 37 °C for 30-120 minutes for gel formation depending on final concentration.

References

1. Bornstein, P. and H. Sage, *Structurally distinct collagen types.* Annu Rev Biochem, 1980. **49**: p. 957-1003.
2. Tanzer, M.L., *Cross-linking of collagen.* Science, 1973. **180**(4086): p. 561-6.
3. Heino, J., *The collagen family members as cell adhesion proteins.* Bioessays, 2007. **29**(10): p. 1001-10.
4. Barczyk, M., S. Carracedo, and D. Gullberg, *Integrins.* Cell Tissue Res, 2010. **339**(1): p. 269-80.
5. Heino, J., *The collagen receptor integrins have distinct ligand recognition and signaling functions.* Matrix Biol, 2000. **19**(4): p. 319-23.
6. Ivaska, J., et al., *Cell adhesion to collagen. Is one collagen receptor different from another?* Vol. 30. 1998. 273-283.
7. Goessler, U.R., et al., *In vitro analysis of integrin expression in stem cells from bone marrow and cord blood during chondrogenic differentiation.* J Cell Mol Med, 2009. **13**(6): p. 1175-84.
8. Ebert, E.C. and A.I. Roberts, *Human intestinal intraepithelial lymphocytes bind to mucosal mesenchymal cells through VLA4 and CD11A.* Cell Immunol, 1996. **167**(1): p. 108-14.
9. Gilcrease, M.Z., *Integrin signaling in epithelial cells.* Cancer Lett, 2007. **247**(1): p. 1-25.