Datasheet for ABIN6952244

Collagen (COL) peptide (Cy3)

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Overview

Quantity:	300 µg
Target:	Collagen (COL)
Source:	Synthetic
Purification tag / Conjugate:	This Collagen peptide is labelled with Cy3.
Application:	BioImaging (BI), Immunofluorescence (IF), Immunohistochemistry (IHC), SDS-PAGE (SDS)

Product Details

Purpose:	Collagen Hybridizing Peptide, Cy3 Conjugate (R-CHP)
Specificity:	CHP binds to the unfolded triple-helical chains of all collagen types (e.g., I, II, III, IV, etc)
Characteristics:	The Collagen Hybridizing Peptide (CHP) is a synthetic peptide that can specifically bind to such denatured collagen strands through hydrogen bonding, both in histology, in vivo, and in vitro (3D cell culture). By sharing the structural motif and the Gly-X-Y repeating sequence of natural collagen, CHP has a strong capability to hybridize with denatured collagen strands, in a fashion that is similar to a DNA fragment annealing to its complimentary DNA strand during PCR. CHP is an extremely specific probe for unfolded collagen molecules: it has negligible affinity to intact collagen molecules due to the lack of binding sites, it is also inert towards non-specific binding because of its neutral and hydrophilic nature. Collagen is the major building block of all load-bearing tissues including tendon, ligament, cornea, cartilage and bone. It was recently found that unfolding of the collagen triple helix can occur during mechanical damage to connective tissues, and that CHP can specifically detect and localize such mechanically unfolded collagen molecules in situ, enabling understanding of the mechanical behavior and damage mechanism of these tissues at the molecular level.

Product Details

	Collagen is also one of the most widely used natural scaffold materials for regenerative medicine. The process of harvesting native extracellular matrix (ECM) by removing cells from animal tissues (i.e., decellularization) may alter the collagen structure and negatively affect the mechanical property and regenerative capacity of the ECM materials. CHP enables assessment of the structural integrity of collagen molecules within these processed matrices, and can facilitate optimization of the decellularization protocols. Additionally, CHP can be used in several biochemical assays, such as in-gel Western blot, for identification and quantification of collagen content in a biological sample. The collagen hybridizing peptide (CHP) is a novel and unique peptide that specifically binds unfolded collagen chains, both in vitro and in vivo. By sharing the Gly-X-Y repeating sequence of natural collagen, CHP has a strong capability to hybridize with denatured collagen chains by reforming the triple helical structure, in a fashion similar to DNA fragments annealing to complementary DNA strands. CHP is extremely specific: it has negligible affinity to intact collagen molecules due to lack of binding sites, and it is inert towards non-specific binding because of its neutral and hydrophilic nature. CHP is a powerful histopathology tool which enables straightforward detection of inflammation and tissue damage caused by a large variety of diseases, as well as tissue remodeling during development and aging. CHP robustly visualizes the pericellular matrix turnover caused by proteolytic migration of cancer cells within 3D collagen culture, without the use of synthetic fluorogenic matrices or genetically modified cells. CHP can measure and localize mechanical injury to collagenous tissue at the molecular level. It also enables assessment of collagen denaturation in decellularized extracellular matrix. In addition, CHP can be used to specifically visualize collagen bands in SDS-PAGE gels without the need for western blot. F-CHP is labeled with fluorescein for direct fluorescence detection.
Purification:	HPLC, MS, Binding Assay, Histology
Purity:	> 95 %

Target Details

Target:	Collagen (COL)
Background:	Collagen is the most abundant protein in mammals. It is the major structural component of

Target Details

	almost all organs and tissues, providing the framework for cell attachment and growth. Programmed collagen degradation occurs during tissue development, homeostasis and repair. However, excessive collagen degradation is implicated in a variety of diseases, such as cancer, inflammation, and fibrosis.The triple helix is the hallmark protein structure of collagen. During tissue remodeling, the triple helical collagen molecules are degraded by specific proteases (e.g., MMP or cathepsin K) and become unfolded at body temperature.
Molecular Weight:	2762.01 g/mol

Application Details

Application Notes:	Straightforward fluorescence detection in red CHP tends to slowly self-assemble into CHP triple helices in solution during storage. Such CHP trimers have no driving force to hybridize with unfolded collagen strands. Therefore, the trimeric CHP must be dissociated to monomers by heating prior to use. Since the trimerization of CHP takes hours to occur at low µM concentrations, the heat-dissociated CHP can stay as active monomer strands that are available for hybridization with unfolded collagen. A common protocol is heating the CHP solution (after diluting to the desired concentration) to 80 °C in a water bath, and quickly quenching it to room temperature followed by immediate application to target collagen substrates, as described below in detail. A heating block and an ice-water bath may be needed in most applications (not provided).
Comment:	• More informative, reliable and convenient than zymography, DQ collagen, SHG, and TEM • High affinity and unparalleled specificity to collagen with essentially no nonspecific binding • Applicable to all types of collagen from all species, relying on collagen's secondary structure instead of any defined sequence for binding • Suitable for both frozen and paraffin-embedded sections with no need for antigen retrieval • A non-antibody approach with no species restrictions against any co-staining antibody • Small size (2% of IgG by MW) enabling facile tissue penetration and whole specimen staining without sectioning • Stable in solution under 4 °C, eliminating the need to aliquot for storage
Reagent Preparation:	Make sure to tap vial down to ensure powder is at the bottom and that it does not fly out upon opening. Dissolve the 0.3 mg of peptide powder in 1 mL of pure water or phosphate-buffered saline (1x PBS), vortex well and centrifuge, to prepare a stock solution containing approximately 100 µM of CHP. Store the stock solution at 4 °C. Dilute the stock solution to assay dependent concentrations upon use. For the 60 µg products, dissolve the powder in 400 µL water or PBS to get a stock solution with a CHP concentration of 50 µM. For the 15 µg samples, dissolve in

Application Details

	100 µL water or PBS for a stock solution with the CHP concentration of 50 µM.
Restrictions:	For Research Use only

Handling

Format:	Powder
Buffer:	PBS, pH 7.4
Storage:	4 °C,-20 °C
Storage Comment:	-20 °C as powder, 4 °C after reconstitution in water

Publications

Product cited in:

Vagnozzi, Maillet, Sargent, Khalil, Johansen, Schwanekamp, York, Huang, Nahrendorf, Sadayappan, Molkentin: "An acute immune response underlies the benefit of cardiac stem cell therapy." in: **Nature**, Vol. 577, Issue 7790, pp. 405-409, (2020) (PubMed).

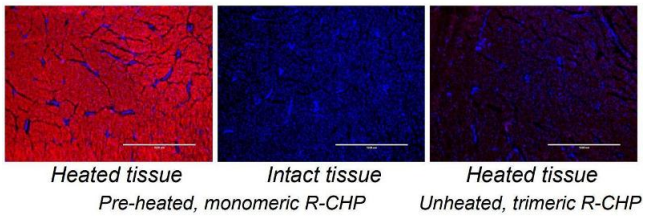
Marino, Converse, Monson, Wriggers: "Molecular-level collagen damage explains softening and failure of arterial tissues: A quantitative interpretation of CHP data with a novel elasto-damage model." in: **Journal of the mechanical behavior of biomedical materials**, Vol. 97, pp. 254-271, (2019) (PubMed).

Abramowitz, Paredes, Zhang, Brightwell, Newsom, Kwon, Custodio, Buttar, Farooq, Zaidi, Pai, Pessin, Hawkins, Fry: "Skeletal muscle fibrosis is associated with decreased muscle inflammation and weakness in patients with chronic kidney disease." in: **American journal of physiology. Renal physiology**, Vol. 315, Issue 6, pp. F1658-F1669, (2019) (PubMed).

Krishnamoorthy, Hoy, Natelson, Torre, Laudier, Iatridis, Illien-Jünger: "Dietary advanced glycation end-product consumption leads to mechanical stiffening of murine intervertebral discs." in: **Disease models & mechanisms**, Vol. 11, Issue 12, (2019) (PubMed).

Feinberg, Zheng, Liu, Wicha, Yu, Weiss: "Divergent Matrix-Remodeling Strategies Distinguish Developmental from Neoplastic Mammary Epithelial Cell Invasion Programs." in: **Developmental cell**, Vol. 47, Issue 2, pp. 145-160.e6, (2018) (PubMed).

There are more publications referencing this product on: Product page



Immunofluorescence

Image 1. Sections of frozen porcine ligament showed drastic differences of R-CHP binding between an intact (middle) and a heat-denatured tissue sample (left). Comparing to the pre-heated, monomeric R-CHP (left), the triple-helical R-CHP used without the pre-heating step (right) exhibited dramatic decrease in binding. Scale bar: 1000 µm. Blue: cell nuclei stained by DAPI.

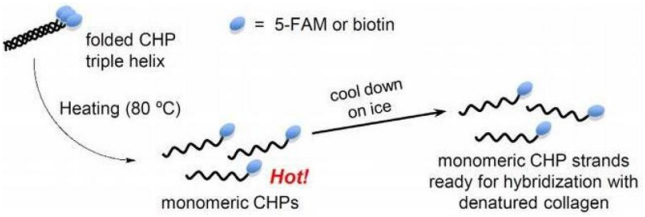


Image 2. CHP tends to slowly self-assemble into CHP triple helices in solution during storage. Such CHP trimers have no driving force to hybridize with unfolded collagen strands. Therefore, the trimeric CHP must be dissociated to monomers by heating prior to use. Since the trimerization of CHP takes hours to occur at low µM concentrations, the heat-dissociated CHP can stay as active monomer strands that are available for hybridization with unfolded collagen.