Damaged Collagen Histological Staining with Collagen Hybridizing Peptides User Guide

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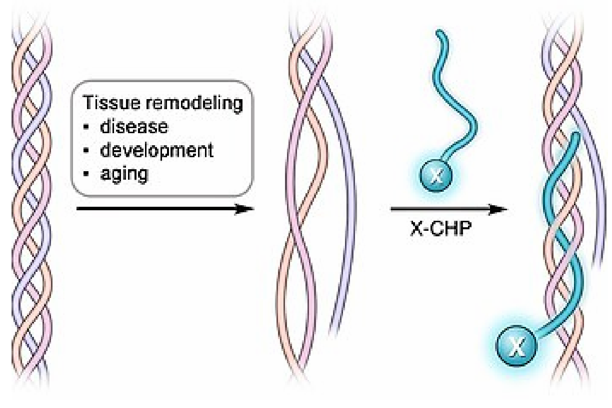
Name	Collagen Hybridizing Peptide, 5-FAM Conjugate	Collagen Hybridizing Peptide, Cy3 Conjugate	Collagen Hybridizing Peptide, Biotin Conjugate
Acronym	**F-CHP**	**R-CHP**	**B-CHP**
Product number	FLU300 / FLU60	RED300 / RED60	BIO300 / BIO60
Specialty	Straightforward fluorescence detection in green	Straightforward fluorescence detection in red	Avidin / streptavidin mediated detection options based on needs, allowing non-green fluorescence and HRP methods to avoid background and enhance signal
Formula	$C_{135}H_{175}N_{31}O_{45}$	$C_{144}H_{198}N_{33}O_{46}S_{2}$	$C_{124}H_{179}N_{33}O_{41}S$
Molecular weight	2952.01 g/mol	3191.44 g/mol	2820.01 g/mol
Ex/Em	494 nm / 512 nm	548 nm / 563 nm	N/A
Synonym	collagen mimetic peptide, CMP		
Applications	Immunofluorescence, immunohistochemistry, SDS-PAGE (in-gel Western blot)		
Solubility	water, aqueous buffers		
Shipping	Shipped as powder at ambient temperature. Store at -20 °C upon arrival and until use.		
Storage	-20 °C as powder for long term storage; 4 °C after reconstitution in water, no need to aliquot and freeze if using within a month. For F-CHP and R-CHP, protect from light.		

Background

Collagen is the most abundant protein in mammals. It is the major structural component of almost all organs and tissues, providing the framework for cell attachment and growth. Programmed collagen degradation occurs during tissue development, homeostasis and repair. The Collagen Hybridizing Peptide (CHP) is a synthetic peptide that can specifically bind to such denatured collagen strands through hydrogen bonding, both in histology [1], *in vivo* [2], and *in vitro* (3D cell culture) [3]. Additionally, CHP can be used in several biochemical assays, such as SDS-PAGE staining, for identification and quantification of collagen content in a biological sample. 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. 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 [4].



Protocol:

Sample reconstitution and handling

Make sure to tap vial down to ensure powder is at the bottom and that it does not fly out upon opening.

1. Dissolve the 0.3 mg of peptide powder (F-CHP, R-CHP, or B-CHP) in 1 mL of pure water or phosphate-buffered saline (1x PBS) and vortex.
   a. This makes a stock solution containing 100 µM of CHP. Store the stock solution at 4 °C. 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.

Ensuring Damaged Collagen

To preserve endogenous damaged collagen in your sample, avoid heating the tissue above 60°C and refrain from any heat-induced epitope retrieval (HIER) or similar thermal processes before applying CHPs. Heating the sample will artificially damage collagen, which will not accurately represent your study conditions. By avoiding heat, you allow CHPs to selectively hybridize with naturally damaged collagen in the sample, ensuring a genuine damaged collagen readout.

Staining histological slides

For tissues that are strongly auto-fluorescent in green, we recommend using B-CHP or R-CHP instead of F-CHP, which allows colorimetric detection, or fluorescence detection in non-green channels.

1. Remove the embedding OCT compound or paraffin with standard procedures.

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2. Since CHP has low non-specific binding to tissue, blocking with serum or BSA can be omitted. However, when co-staining samples with CHP and antibodies, we recommend blocking the tissue slides with 10% serum or 5% BSA. (For certain tissue types, e.g., kidney, it may be necessary to block endogenous biotin using a standard kit for B-CHP staining.)
3. Dilute the CHP stock solution in a PBS buffer. A concentration of 20 μM is recommended for initial trials. [Note: the optimal CHP concentration (usually 5-30 μM) is sample dependent.] Depending on the sample size, one tissue section may require 20 to 200 μL of diluted CHP solution.
4. Using a heating block or a water bath with temperature control, heat the dilute CHP solution in a sealed microtube at 80 °C for 5 min. (There is no need to heat the CHP stock solution.)
5. To avoid thermal damage to the tissue sample, after heating, immediately immerse the CHP microtube in an ice-water bath for 15-90 s to quench the solution to room temperature. The required cooling time depends on the solution volume. The microtube can be quickly centrifuged to collect condensation in the tube. Subsequently pipet the solution onto each tissue sample quickly. Achieving minimal deadtime (~1-3 min) is encouraged for this step. (Note: other staining agents, such as a primary antibody, can be diluted into the quenched CHP solution for co-staining.)
6. Incubate the tissues with the staining solution at 4 °C for 2 h. For optimal results, overnight incubation is recommended.
7. After staining, wash the tissue slides in PBS for 5 min three times at room temperature.
8. Samples stained with F-CHP and R-CHP can be analyzed with a standard fluorescence microscope using the GFP and RFP channel/filters. For tissues treated with B-CHP, the collagen-bound CHP can be detected by an avidin/streptavidin-mediated method. [Note: To detect B-CHP, we recommend incubating the tissue samples with 0.005 mg/mL of a streptavidin conjugate (e.g., AlexaFluor dyes labeled streptavidin) in a PBS solution containing 1% BSA for 1 h at room temperature. To detect the co-stained primary antibody, a labeled secondary antibody can be either diluted into the streptavidin solution (for B-CHP costaining) or added to the slides directly after dilution in a PBS solution containing 1% BSA (for co-staining with F-CHP or RCHP).]

References

[1] *In situ* imaging of tissue remodeling with collagen hybridizing peptides. *ACS Nano*, **2017**, 11, 9825–9835.

[2] Targeting collagen strands by photo-triggered triple-helix hybridization. *Proceedings of the National Academy of Sciences of the United States of America*, **2012**, 109, 14767–14772.

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SDS-PAGE/In-Gel Western with Collagen Hybridizing Peptides User Guide

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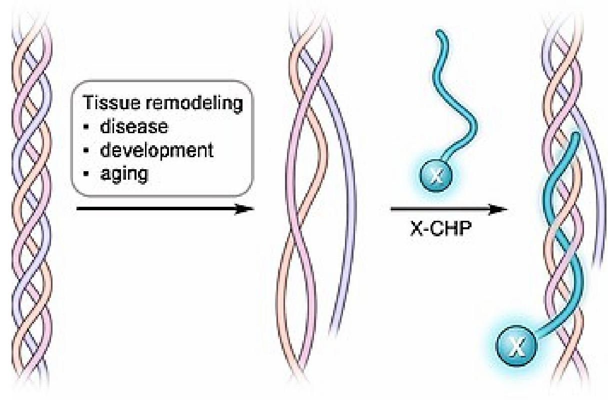

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   a. This makes a stock solution containing approximately 100 μM of CHP. Store the stock solution at 4 °C. For the 60 μg products, dissolve the powder in 400 μL water or PBS to get a stock solution with a CHP concentration of 50 μM.

Staining an in-gel Western blot

1. Heat collagen proteins at 70 °C in an SDS buffer and resolve the protein bands by an SDS-PAGE gel (e.g., 4-12% bis-tris gel). It is recommended to add 0.5-2 μg of collagen into each protein lane for strong signals.
2. After electrophoresis, wash the PAGE gel by deionized water for 5 min three times to remove the remaining SDS. Fixation of protein bands is not required. An area of interest can be cut out of the gel to reduce CHP usage in staining.
3. Prepare a dilute solution of 1-6 μM F-CHP / R-CHP from the stock. Heat the dilute solution (1-5 mL) at 80 °C for 5-10 min, and immediately add it onto the cropped PAGE gel to soak it in CHP solution.
4. At room temperature, stain the gel in F-CHP / R-CHP solution in a small staining chamber under gentle shaking for 3 h in dark, followed by washing with water at least three times (0.5 h each time) to remove unbound CHP.
5. The stained collagen bands can be visualized using a fluorescence gel imager/scanner. The gel can be further stained with coomassie brilliant blue.

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References

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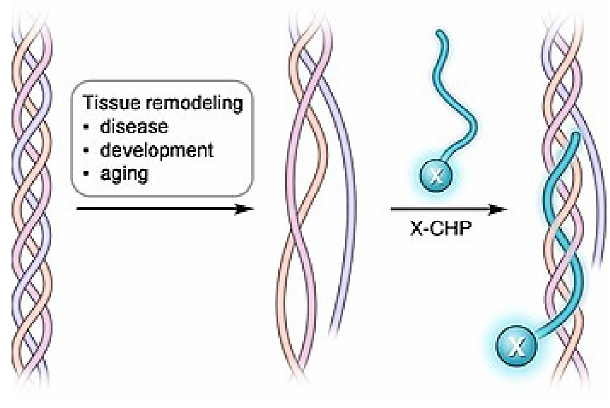

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Ensuring Total Collagen

To measure total collagen, the endogenous collagen in the sample must be artificially damaged using a heat-induced epitope retrieval (HIER) or an alternative heating method, such as a tissue steamer or microwave. This process disrupts the collagen's triple helix structure, allowing CHPs to bind. This controlled, uniform damage enables a reliable readout of total collagen in the sample.

Staining histological slides

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