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Technical Data Sheet

For research use only
Not intended or approved for diagnostic or therapeutic use.

Product: **Collagen Hybridizing Peptide *In Vivo* Probe**

Name	Collagen Hybridizing Peptide, sulfo-Cyanine-7.5 conjugates
Acronym	**sCy7.5-CHP *In vivo* Kit**
Unit size (Kit)	24 nmole (10 doses) Targeted, 8 nmole (3 doses) Control
Unit size (single vial)	8 nmole (3 doses) This is for a single vial of either the targeted or control peptides.
Specialty	Straightforward, near-infrared fluorescence, *in vivo* imaging of collagen remodeling in small animals
Formula	$C_{153}H_{190}F_9N_{30}O_{49}S_4$
Molecular weight	3703.72 g/mol
Wavelengths	778 nm / 797 nm (Excitation / Emission)
Synonym	collagen mimetic peptide, CMP; collagen-like peptide, CLP
Purity	> 90% by HPLC
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. Protect from light.

Background

sCy7.5-CHP contains a novel collagen hybridizing peptide (CHP) sequence labeled with a near-infrared fluorophore (Sulfo-Cyanine-7.5). The CHP peptide is designed to specifically bind to denatured collagen chains in tissues under disease and injury, while the sulfo-Cyanine7.5 dye allows straightforward *in vivo* imaging in small animals using a standard near-infrared fluorescence imager [1]. Unlike 3Helix's other CHP products for *in vitro* uses (e.g., B-CHP and F-CHP), the novel CHP sequence in this product has been optimized for facile *in vivo* imaging. It contains a non-natural amino acid residue which reduces self-hybridization of the CHP peptide [1]. Therefore, unlike B-CHP (BIO60/300) and F-CHP (FLU60/300), sCy7.5-CHP can be directly injected into live animal models for collagen hybridization ***without*** the pre-heating step before use. This enables easy, accurate, and reliable imaging of denatured collagen *in vivo*.

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. However, excessive collagen degradation is implicated in a variety of diseases, such as cancer, inflammation, and fibrosis [2].

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. The Collagen Hybridizing Peptide (CHP) is a synthetic peptide that can specifically bind to such denatured collagen strands through hydrogen bonding, both in histology [3], *in vivo* [1], and *in vitro* (3D cell culture) [1]. 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 [2,3,4]. 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 [5].

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 [6], and that CHP can specifically detect and localize such mechanically unfolded collagen molecules *in situ* [6], enabling understanding of the mechanical behavior and damage mechanism of these tissues at the molecular level.

Applications: *In vivo* imaging in small animals for research purposes

Suggested Protocol for *In Vivo* Imaging

Note: all concentrations and directions for *in vivo* use of sCy7.5-CHP are based on use in mouse models. When using larger animals (rat, guinea pig, etc.) the dose, concentration, and administration route may need to be adjusted. The ex/em of the sulfo-cyanine 7.5 probe is 778/797 nm.

This kit contains a Targeted and Control CHP. The Control CHP is a peptide containing the same amino acid composition as the Target CHP, but the sequence order is scrambled. This will act as a negative control and will not bind to denatured collagen. The scrambled sequence inhibits hybridization into a triple-helix because it does not have a glycine residue in every third position, which is required for folding.

(A) Sample reconstitution and handling

A.1 Reconstitution for *in vivo* Kit- Please follow these instructions if you purchased the *in vivo* kit, disregard section A.2.

sCy7.5-CHP Targeted: Dissolve the peptide powder (24 nmole, 89 µg) in 1.2 mL (1200 µL) of phosphate-buffered saline (1x PBS), vortex well and centrifuge briefly, this will yield a stock solution containing approximately 20 µM of sCy7.5-CHP. Next, move the 1.2 mL solution containing the sCy7.5-CHP to a vial or tube that can hold at least 3 mL before adding another 1.2 mL of 1x PBS. This will bring the total volume to 2.4 mL and the final concentration to 10 µM of sCy7.5-CHP which is suitable for injection. This volume is enough for 10 doses (injections) with an extra 400 µL to account for solution lost in the dead volume in syringes or pipetting errors.

sCy7.5-CHP Control: Dissolve the peptide powder (8 nmole, 30 µg) in 800 µL of 1x PBS, vortex well and centrifuge briefly to prepare the final stock solution containing approximately 10 µM of sCy7.5-CHP Control. We recommend preparing the stock solution freshly before animal dosing however, the used CHP solution can be stored at 4 °C for up to one month for future experiments. This volume is enough for 3 doses (injections) with an extra 200 µL to account for solution lost in the dead volume in syringes or pipetting errors.

A.2 Reconstitution for Individual Vials- If you purchased an individual vial of the sCy7.5-CHP (targeted or control) please follow these instructions and disregard the instructions in A.1 above.

Dissolve the peptide powder (8 nmole, 30 µg) in 800 µL of 1x PBS, vortex well and centrifuge briefly to prepare the final stock solution containing approximately 10 µM of sCy7.5-CHP peptide. We recommend preparing the stock solution freshly before animal dosing however, the used CHP solution can be stored at 4 °C for up to one month for future experiments. This volume is enough for 3 doses (injections) with an extra 200 µL to account for solution lost in the dead volume in syringes or pipetting errors. Alternatively, you can make 400 µL stock solution of 20 µM and inject 100 µL as a single bolus of less volume if you have certain injection guidelines.

(B) *In vivo* injection and imaging

Note: Details of protocol (B) is discussed in ref [1].

1. We recommend 2 nmole of sCy7.5-CHP for injection into each mouse. Draw 200 µL of the 10 µM stock solution into sterile syringe, and make sure to carefully remove any bubbles from the syringe before injection.
2. Inject 1 dose (200 µL) of sCy7.5-CHP *via* the tail vein. The mouse can be anesthetized (i.e. isoflurane) or simply restrained during injection.
3. After injection, the mouse can be imaged on a near-infrared fluorescence imager with the appropriate filter and channel for the sCy7.5 fluorophore (e.g., 800nm, sCy7.5) at any time point depending on the research question being addressed (30 min up to several days).

Recommendation and Expected Results:

- Typically, a time point between 3 and 12 hrs post injection, the image shows clear accumulation in normal skeletal tissues with good signal-to-noise ratio. Images acquired at time points earlier than 3 h will typically show high signal intensity over the entirety of the animal and image adjustments (exposure time, control signal subtraction, thresholding, etc.) may be required in order to see a difference between regions with higher-than-normal collagen turnover.
- The fluorescent signal intensity typically drops considerably after 72 h with a 4 nmole injection, and the signal intensity may remain in the targeted tissues (e.g., spine) for days post tail-vein injection. However, injecting a lower dose (2 nmole), it is expected to have faster clearance.
- For detailed imaging of the skeletal system or organs, we highly recommend removing the skin and performing *ex vivo* examination post-mortem.
- The concentrations and dosing volumes are only a recommendation, and both may need to be altered to fit your specific

animal model. Customers have found success with doses ranging from 1 nmole up to 4 nmole. Additionally, the suggested imaging times may vary and should be optimized for your specific application.

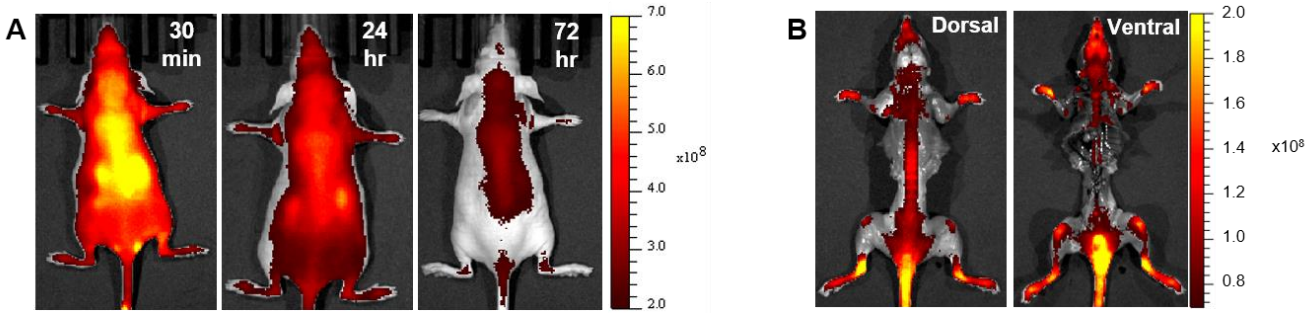

Figure 1. Representative *in vivo* and *ex vivo* images after i.v. injection of 4 nmole of sCy7.5-CHP in a nude mouse. (**A**) *In vivo* images at 30 min, 24 h, and 72 h post tail-vein injection. (**B**) Representative *ex vivo* images for examining signal intensity in the spine and joints. Similar *in vivo* results have been reported in references [1] and [4].

References

[1] Visualizing collagen proteolysis by peptide hybridization: From 3D cell culture to *in vivo* imaging. *Biomaterials*, **2018**, 183, 67–76.

[2] Targeting and mimicking collagens via triple helical peptide assemblies. *Current Opinion in Chemical Biology*, **2013**, 17, 968–975.

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

[4] 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.

[5] Direct detection of collagenous proteins by fluorescently labeled collagen mimetic peptides. *Bioconjugate Chemistry*, **2013**, 24, 9–16.

[6] Molecular level detection and localization of mechanical damage in collagen enabled by collagen hybridizing peptides. *Nature Communications*, **2017**, 8, 14913, doi: 10.1038/ncomms14913.