

## <sup>125</sup>I-Radiolabeling of Secreted Frizzled-Related Protein-1

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### **Background**

Secreted frizzled-related proteins (sFRP) comprise a family of secreted glycoproteins (sFRP1, sFRP2, sFRP3, sFRP4, sFRP5) in humans known to modulate Wnt-dependent signal transduction.<sup>1</sup> Approximately 300 amino acids in length, sFRP-1 molecules contain a cysteine-rich domain (CRD) homologous to the putative Wnt-binding site of the Frizzled (Fzd) family, a set of seven-pass transmembrane cell surface receptors for Wnts.<sup>2</sup> While the normal Wnt pathway functions in embryonic development and tissue homeostasis, dysregulation of Wnt signaling has been implicated in a number of human malignancies.<sup>3</sup> Given the potential involvement of these proteins in a number of biological processes, including tumor suppression, a more rigorous understanding of their binding interactions is warranted. In the work described herein, sFRP-1 was radiolabeled with I-125, a gamma energy radioisotope with a 60 day half-life, for use in a variety of biological assays.

### **Chemistry**

The radioiodination of Secreted Frizzled-Related Protein-1 (sFRP-1) was performed using Chloramine-T as an oxidant.<sup>2</sup>

### **Experimental**

**General.** All chemicals were purchased from Sigma Aldrich except where noted. Sodium [<sup>125</sup>I]iodide (17.4 Ci/mg) in NaOH (1.0x10<sup>-5</sup> M) was supplied by PerkinElmer. FRP-1 was provided by Dr. Jeffery Rubin from NCI. MALDI-TOF mass spectrum was recorded on a Shimadzu Biotech Axima-CFP Plus spectrometer. A supersaturated solution of dihydroxybenzoic acid (DHB) (50% CH<sub>3</sub>CN, 50% water with 0.1% trifluoroacetic acid) was

used as a matrix for MALDI-TOF analysis of sFRP-1.  $m/z$  (MALDI-TOF) of sFRP-1 was found 35346Da. Radio-TLC scan was performed on an AR-2000-TLC imaging scanner.

**<sup>125</sup>I-Radiolabeling of sFRP-1.** FRP-1 (7 $\mu$ L, 1.5mg/mL in PBS buffer, pH 7.4), sodium phosphate buffer (20 $\mu$ L, 0.5M, pH 7.4) and sodium [<sup>125</sup>I]iodide solution (10  $\mu$ L, 37MBq, 3.7GBq/mL) were transferred into a v-vial. The reaction was initiated by adding Chloramine-T solution (20 $\mu$ L, 1mg/mL in 0.5M phosphate buffer) and shaken for 45 seconds. After that, sodium sulfite (100 $\mu$ L, 1mg/mL in PBS buffer) was added to the reaction and continuously shaken for 2 minutes. The reaction mixture was loaded on a PD-10 column and eluted using 0.05M phosphate buffer with 1% BSA. The fractions containing radioiodinated sFRP-1 were combined. The radiochemical purity (over 98%, Figure 1) was detected by radio-TLC scanner. The radiochemical yield of 73.9% with specific activity 42.8 $\mu$ Ci/ $\mu$ g was obtained.

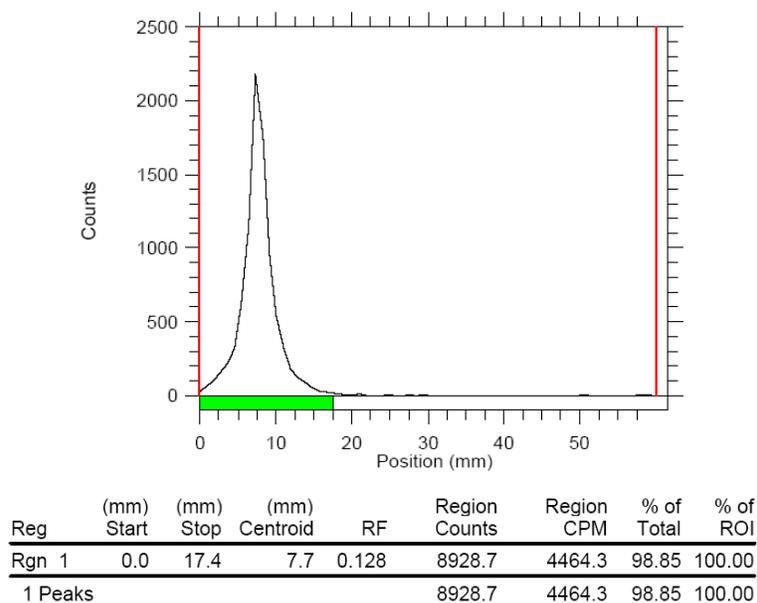


Figure 1. Radiochemical purity of [<sup>125</sup>I] sFRP-1

## References

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