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Technical Brief

Recombinant Human Intrinsic Factor

Recombinant Human Intrinsic Factor (IF) from Scripps Laboratories is offered as a high-quality replacement for native porcine IF. The quality of the native porcine IF available to the clinical diagnostic industry has declined in recent years, so Scripps Laboratories developed a highly purified recombinant IF that is a reliable and economical alternative to native IF. Data are presented here to demonstrate the suitability of Recombinant Human Intrinsic Factor for research use or Vitamin B12 assay development.

SDS-PAGE

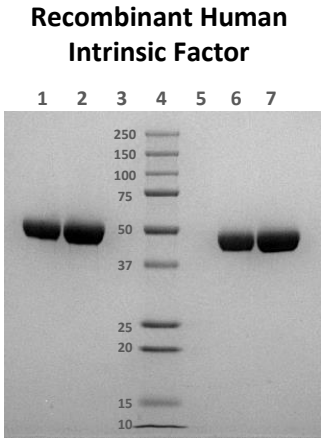
Figure 1 presents an SDS-PAGE image of highly purified Recombinant Human IF, Mat. No. I0821-90658. It displays an approximate molecular weight greater than 40kDa and less than 50 kDa. This is consistent with the reported molecular weight of 44-48 kDA for Native Human IF.[1] In addition, the purified product shows no visible contaminating proteins, both in the reduced/heated (lanes 1,2) and non-reduced/not heated (lanes 6,7) samples.

1. Allen and Mehlman. J. Biol. Chem., 1973, (248): 3660.

ISOELECTRIC FOCUSING

Recombinant Human IF is a glycosylated protein, so we employed Isoelectric Focusing to visualize the heterogeneity of the glycosylation patterns. Figure 2 shows a variety of distinct isoelectric points, ranging from 4.75 to 6.3, demonstrating the microheterogeneity of this purified, glycosylated protein. The Recombinant Human IF sample was run in duplicate.

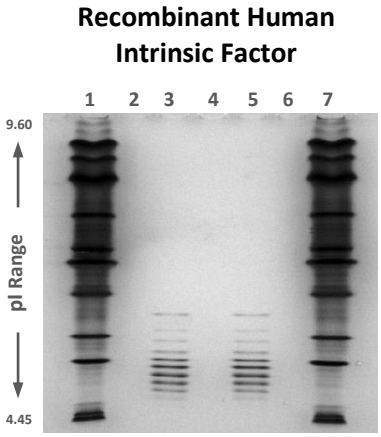
SDS-PAGE



Lanes
1 Reduced/Heated – 5 µg
2 Reduced/Heated – 10 µg
3 Blank
4 MW Markers (kDa)
5 Blank
6 Non-Reduced/Not Heated – 5 µg
7 Non-Reduced/Not Heated – 10 µg

Figure 1. SDS-PAGE of Recombinant Human Intrinsic Factor under reduced/heated (lanes 1,2) and non-reduced/not heated (lanes 6,7) conditions.

ISOELECTRIC FOCUSING



Lanes
1 pI Markers
2 Blank
3 Recombinant Human IF
4 Blank
5 Recombinant Human IF
6 Blank
7 pI Markers

Figure 2. Isoelectric Focusing of Recombinant Human Intrinsic Factor, run in duplicate on a pH 3-10 IEF gel (lanes 3,5).

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BIOTIN CONJUGATION & VITAMIN B12 ELISA

Recombinant Human IF and Native Porcine IF were conjugated to biotin and each conjugate was used side-by-side as a component of a Vitamin B12 ELISA.[2]

Figure 3 shows the generation of standard curves, using the Vitamin B12 standards included with the ELISA kit. The top graph was prepared using the Recombinant Human IF-Biotin conjugate, while the bottom graph was prepared with the Native Porcine IF-Biotin conjugate. (Note: The Native Porcine IF-Biotin used is the reagent included with the ELISA kit.) The charts demonstrate good correlation, indicating Recombinant Human IF-Biotin is suitable for use in generating a standard curve in ELISA.

Figure 4 presents a scatter plot showing good correlation when Recombinant Human IF-Biotin is substituted for Native Porcine IF-Biotin in the Vitamin B12 ELISA. The data represent the recoveries of a series of 36 Vitamin B12 samples with both biotin conjugates. With an R-squared (R^2) value of 0.9979, the linear regression shows a strong positive correlation between Recombinant Human IF and Native Porcine IF.

Of note, Recombinant Human IF-Biotin demonstrated good thermal stability after storage at 37°C for 2 weeks.

2. Assay data generated by Calbiotech - El Cajon, CA USA.

Vitamin B12 Standard Curve Preparation

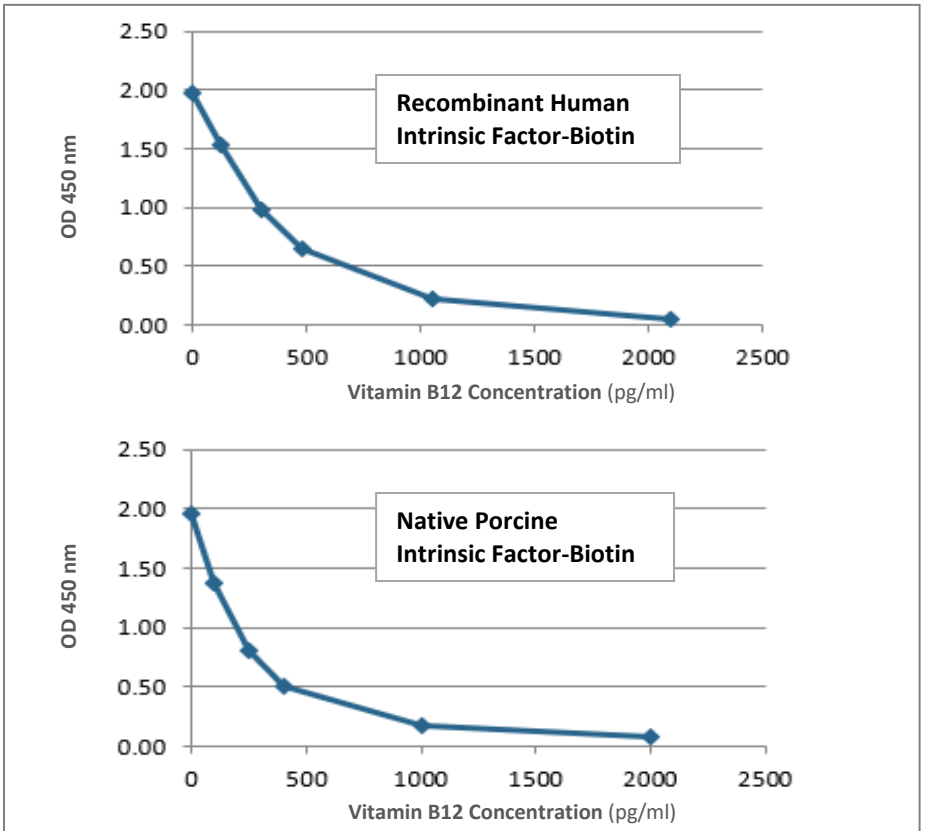

Figure 3. Analysis of Vitamin B12 assay standards using Recombinant Human IF-Biotin and Native Porcine IF-Biotin conjugates. Similar standard curves were prepared with each conjugate.

Vitamin B12 Sample Recovery

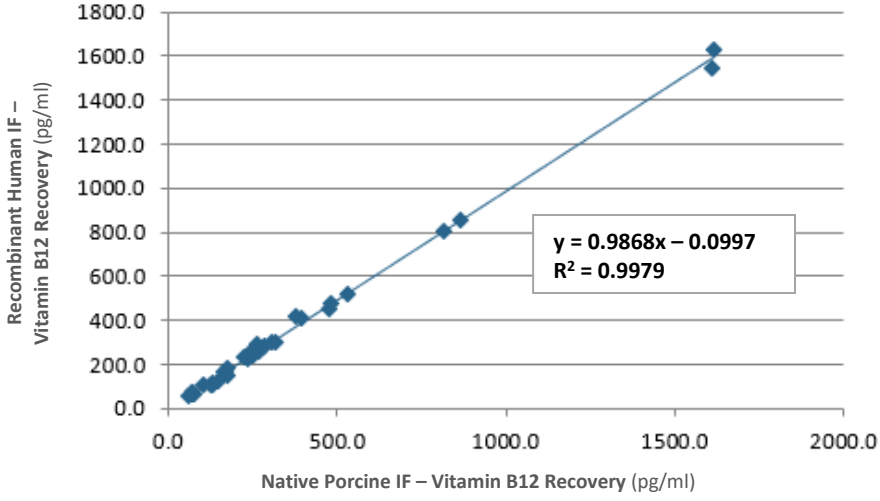

Figure 4. Linear regression analysis of Vitamin B12 sample recovery using Recombinant Human IF-Biotin and Native Porcine IF-Biotin conjugates in a Vitamin B12 ELISA.

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HPLC

Analyzed by HPLC, the elution profile of Recombinant Human IF in Figure 5 displays a single peak with an elution time of 17.092. The absence of any minor peaks confirms what is corroborated by SDS-PAGE: Recombinant Human IF is a highly purified product with no discernable contaminants.

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The data presented here for Recombinant Human IF demonstrate it possesses the physical characteristics and Vitamin B12-binding capabilities to make it a suitable replacement for Native Porcine IF in a Vitamin B12 assay. It is a highly purified, glycosylated protein and, as a renewable recombinant product, it is ideal for research work as well as long-term use in the development and production of a commercial assay.

Recombinant Human Intrinsic Factor is in stock and available now. Use the link at right to learn more.

HPLC

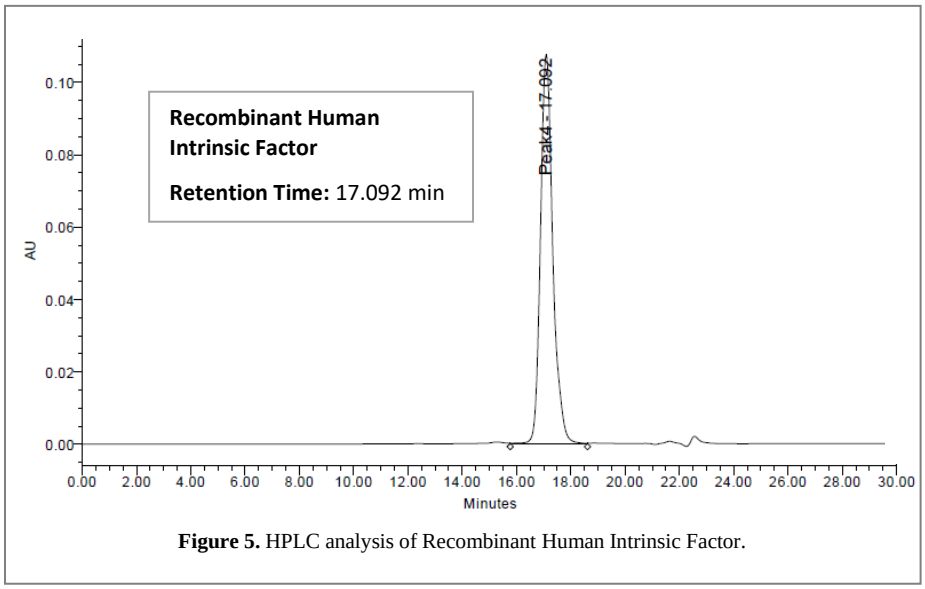

Figure 5. HPLC analysis of Recombinant Human Intrinsic Factor.

Ordering Information

Product Description	Cat. No.	Part No.	
Recombinant Human Intrinsic Factor	I0821	90658	*View I0821*

Expressed Without Affinity Tags

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