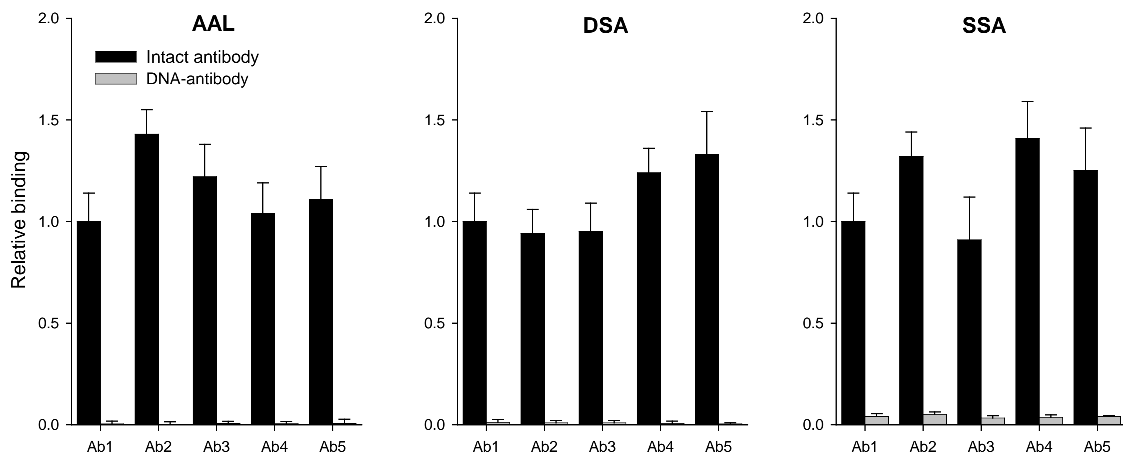
Supplemental Table S1 DNA sequences for DNA tags, DNA array probes and shrNA

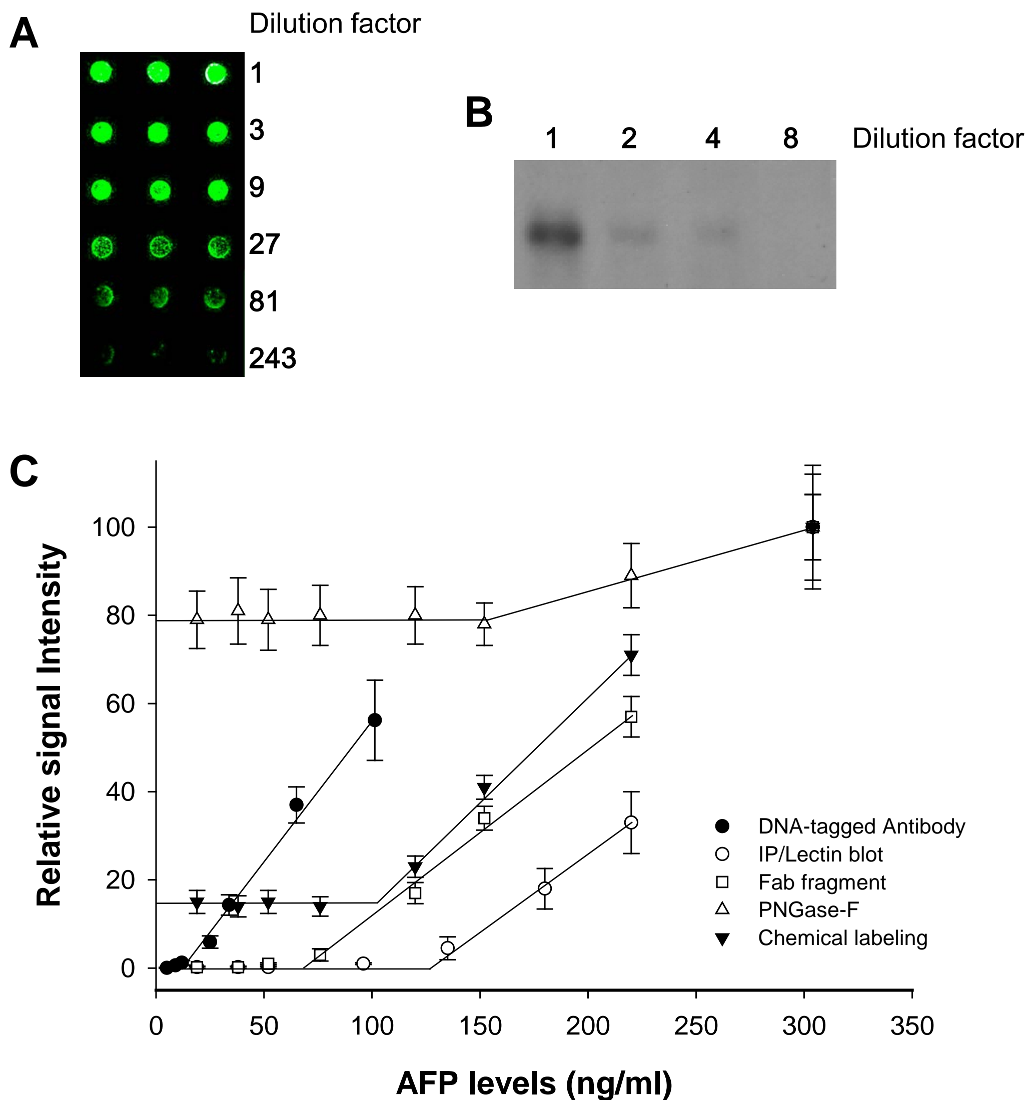
		5'-3' sequence
T7 promoter		Hydrazine-ATGGAATTCCTAATACGACTCACTATAGGG
DNA-tag sequence	AFP	GTTCTCATAAAATTGCCGCCGGTCCATAGCTTTTTTTTTT-Biotin
	HPX	ATTACCACCCCTATCTCACCGAGTTCGATGTTTTTTTTTT-Biotin
	A2M	GTATTCTAATGCACCCAACCCTGAGCGTCTTTTTTTTTTT-Biotin
DNA microarray probes sequence	AFP	GCTATGGACCGGCGGCAATTTTATGAGAAC
	HPX	CATCGAACTCGGTGAGATAGGGGTGGTAAT
	A2M	AGACGCTCAGGGTTGGGTGCATTAGAATAC
FUT8 shRNA		CCGGGTCTATAATGACGGATCTATACTCGAGTATAGATCCGTC ATTATAGACTTTTTG



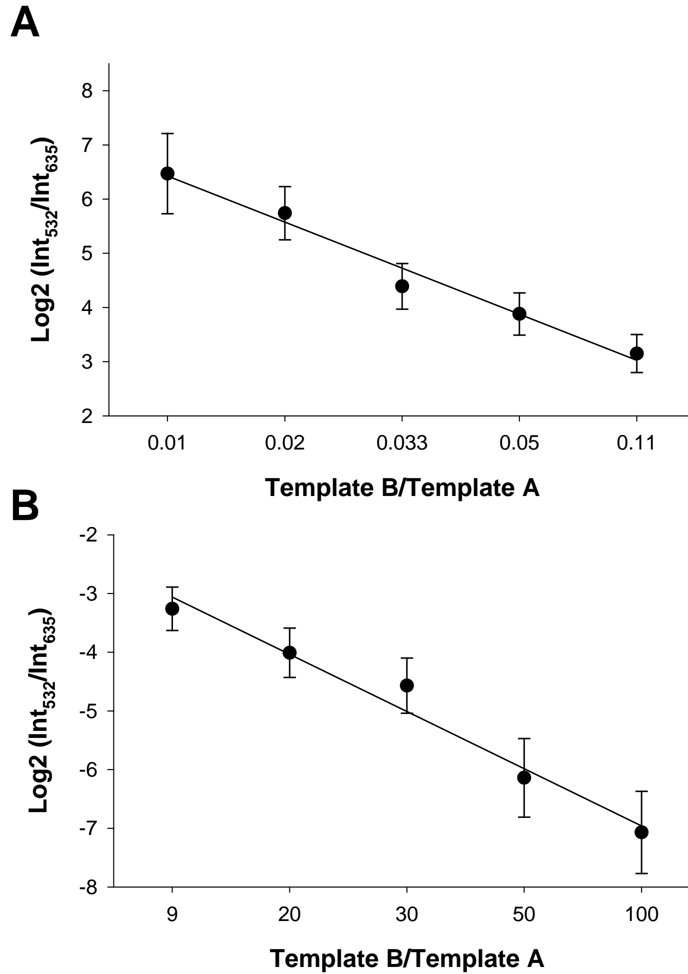
Supplemental Figure S1. Pre-clearing of an interference fraction of antibody with lectin-bound beads. In Fig. 2D, a fraction of DNA-tagged antibodies was not bound to AAL, DSA, and SSA. It indicates that those fraction surely interferes with precise and reproducible measurement of specific glycoforms, and thus should be removed from the antibody pool. To this end, DNA-tagged antibodies were pre-cleared with either AAL, DSA, or SSA-bound beads. The result shows that DNA-tagged antibodies can be used to detect AAL- or DSA-bound glycoforms of biomarkers after the precedent pre-clearing step. Because most commercially available antibodies are not quality-controlled with respect to the bound N-glycans, the pre-clearing step should be accompanied for general uses of a wide range of lectins. It should be, however, noted that terminal sialic acid-binding lectins, such as SSA, are not applicable for our method. It appears that the DNA binding does not affect to the lectin-binding properties of terminal sialic acids.

Supplemental Table S2 Derivation of an average coefficient of variation (CV) value

Protein (μg)	0.5	1	2	3	5	10
Fluorescence Intensity ($X10^4$)	0.34	0.84	2.61	3.41	6.91	13.4
	0.31	0.79	2.33	2.94	6.33	12.7
	0.29	0.75	2.37	2.99	6.78	13.1
	0.39	1.11	2.77	3.05	6.45	12.9
	0.41	0.73	2.45	3.74	7.43	14.3
	0.43	0.82	2.91	3.88	7.02	15.1
	0.33	0.87	2.67	3.48	7.23	12.4
	0.37	0.84	2.23	3.65	6.42	13.9
	0.39	0.89	2.19	3.42	6.88	13.7
Mean	0.36	0.85	2.50	3.39	6.83	13.5
Stand. Dev	0.047	0.111	0.249	0.339	0.376	0.850
CV (%)	13.08	13.08	9.98	9.97	5.50	6.30
Ave. CV (%)	9.65					



Supplemental Figure S2. Confirmation of Improved sensitivity of the DNA-tagged antibody-based approach (A) compared to the immunoprecipitation-lectin blot modality (B) and other possible modalities (C) in serum. A serum specimen from an HCC patient was used to implement the comparison of analytical sensitivity of both modalities. An ELISA test revealed an AFP level of 304 ng/ml for the specimen. The serum was diluted as indicated, and subjected to measurement of fluorescence intensity (A) and band intensity on an X-ray film (B). The band intensity in B was calculated from the digitalized, scanned files using ImageJ software (http://rsbweb.nih.gov/ij/). The extrapolation revealed a *ca*. 87-fold increment in the analytical sensitivity. (C) The other possible modalities for quantification of a glycoform including ELISA test using Fab fragment antibody as capture antibody, ELISA test using PNGase-F-treated capture antibody, and chemical labeling methods were also compared. The result indicate that our method showed a far higher analytical sensitivity compared to the other modalities. ELISA tests using PNGase-F treated capture antibody and chemically labeled capture antibody showed significantly high threshold values, which is thought to arise from the incomplete enzymatic cleavage and chemical binding reactions. The presented figures are representative ones from triplicate experiments.

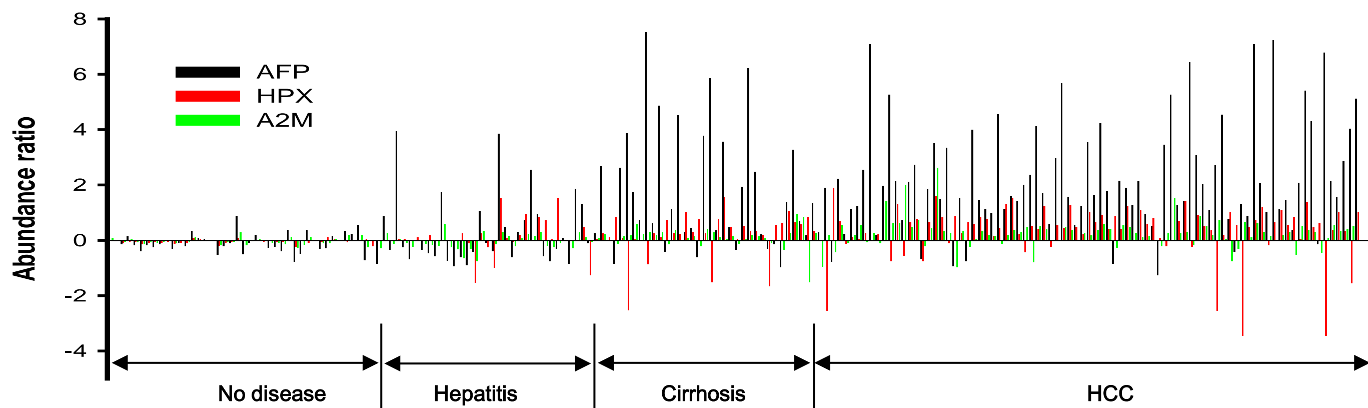


Supplemental Figure S3. Standard curves for external ranges of 1:100-1:9 (A) and 9:1-100:1 (B). The standard curve obtained in a range of 1:9 to 9:1 was not applicable to both outer extreme ranges. Test values either exceeding or below the range of the standard curve in Fig. 5E was fitted to these additional standard curves. The results show that a linearity was also secured in the individual curve, and biomarkers with significantly high or low glycoform levels can be analyzed with analytical validity.

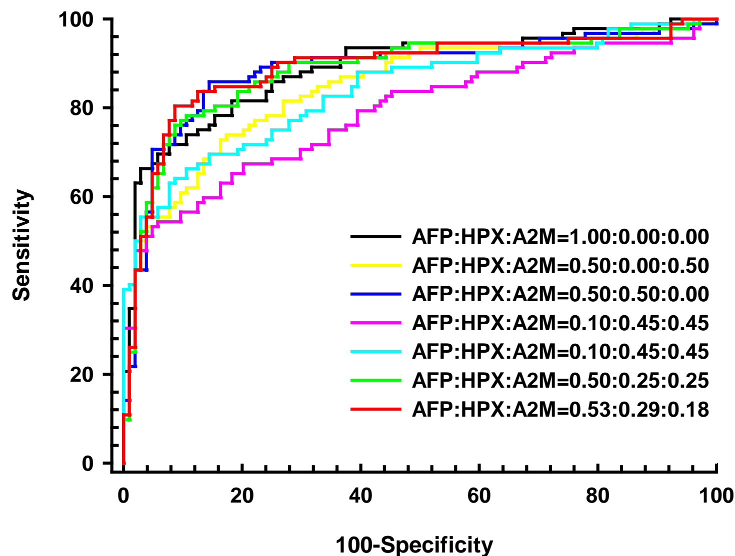
Supplemental Table S3 Baseline characteristics of reference specimens

	Control 1	Control 2	Control 3
Age	56	54	59
Sex	M	M	F
Etiology			
HBV	-	-	-
HCV	-	-	-
Alcohol	-	-	-
Anemia	-	-	-
Inflammation	-	-	-
AFP level	3.7 ng/ml	3.3 ng/ml	2.6 ng/ml
Pooled level[a)]		3.4 ng/ml	
HPX level	713 μg/ml	811 μg/ml	734 μg/ml
Pooled level[a)]		769 μg/ml	
A2M level	2.4 mg/ml	2.6 mg/ml	2.5 mg/ml
Pooled level[a)]		2.5 mg/ml	

a) Each value is a mean of three replicative ELISA tests.

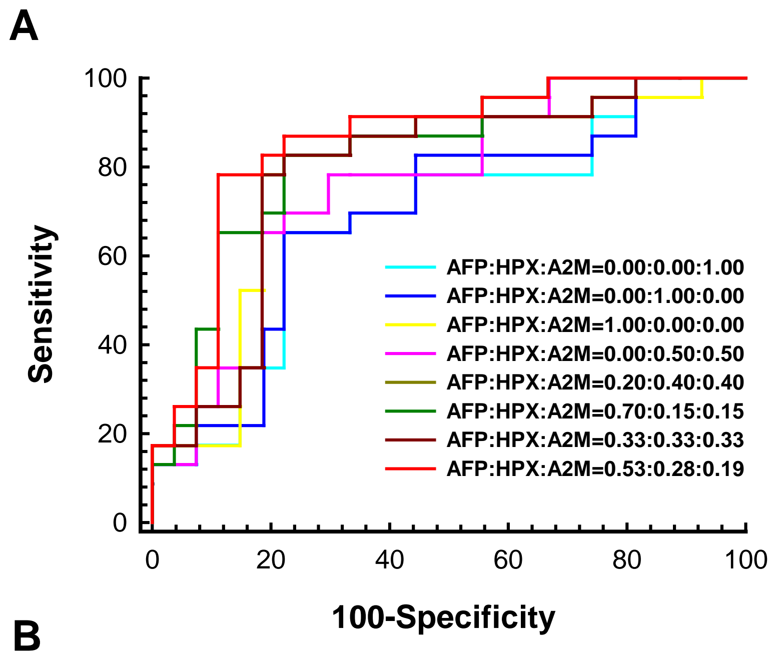


Supplemental Figure S4. The abundance ratios of AFP, HPX, and A2M for 196 specimens. The normalized levels of fuco-form was calculated by subtracting the Log2 ratio of biomarker levels as seen in equation 1. The abundance ratios of the triple biomarkers were obtained from ELISA tests.



Supplemental Figure S5. Comparison of the ROC curves according to varied weight values. The overall diagnostic fuco-index (I_f) was derived from the sum of each weighted fuco-form levels of AFP, HPX, and A2M. As the weight values are varied, the ROC curves showed different patterns, and the AUROC values changed in a range of 0.693-0.889. When AFP was not weighted, the AUROC showed the lowest value. The highest AUROC value (0.889) was obtained in weight values of 0.53/0.29/0.18 for AFP, HPX, and A2M, respectively.

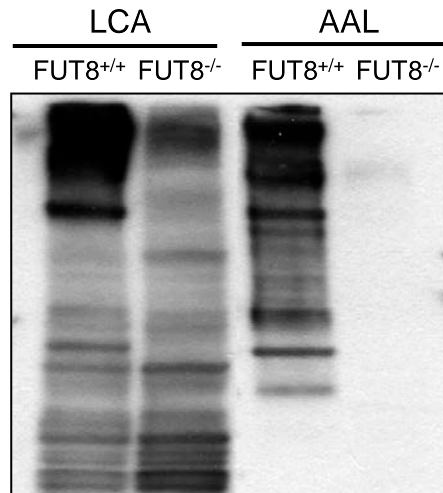
A



B

AFP:HPX:A2M	Sensitivity (%)	Specificity (%)	AUROC
0.00:0.00:1.00	65.2	77.8	0.723
0.00:1.00:0.00	65.2	77.8	0.738
1.00:0.00:0.00	82.6	77.8	0.804
0.00:0.50:0.50	73.9	77.8	0.761
0.20:0.40:0.40	78.3	81.4	0.818
0.70:0.15:0.15	82.6	77.8	0.823
0.33:0.33:0.33	78.3	81.4	0.818
0.53:0.28:0.19	82.6	81.4	0.857

Supplemental Figure S6. Comparison of diagnostic performance of fuco-index (I_f) according to varied weight values in an independent test set. (A) The overall diagnostic fuco-index was derived from the same formula applied to the training set, and the resultant ROC curves were drawn. Because the number of specimens in this test set was relatively small, the diagnostic performance was identical in a wide range of the weight value for AFP. That is, the sensitivity and specificity were identical in the weight value range of 0.4-0.6 for AFP. However, the highest AUROC value (0.857) was also obtained in weight values of 0.53, 0.29, 0.18 for AFP, HPX, and A2M, when compared with that of other weight values. (B) Sensitivity, specificity, and AUROC values were shown for each weight value. The pre-built weight value of 0.53, 0.28, and 0.19 for AFP, HPX, and A2M also yielded the highest diagnostic performance in this test set.



Supplemental Figure S7. AAL as a preferred lectin probe for fuco-forms of a biomarker. Conditioned media of parental and Fut8 knock-out cells were retrieved and total secreted proteins were resolved on a 10% SDS-PAGE gel. A lectin blot analysis was performed using AAL and LCA. The result shows that the use of LCA as a probe is not preferred for at least our method, because it does not clearly differentiate between fuco- and non-fucoforms. This may be explained by the binding properties of LCA, which can bind to non-fucosylated glycoforms with a lower affinity than to fucosylated glycoforms. However, AAL was found to be adequate for our platform because the lectin bound exclusively to fuco-forms, which is the reason why we adopt AAL for measurement of fuco-forms.