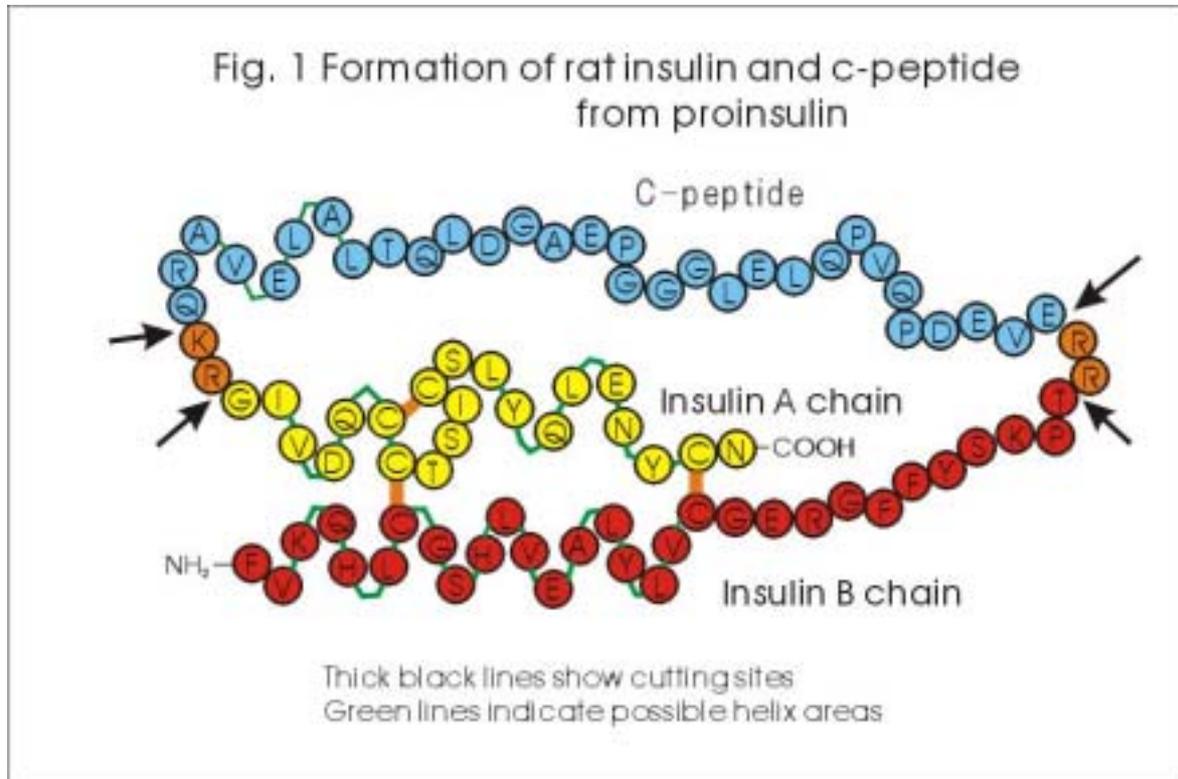


## Insulin ELISA Kit S-type and Proinsulin

### What is proinsulin?

The amino acid sequence of proinsulin produced in B-cells of pancreatic islet of Langerhans is shown in the following figure (in the case of rat).



This proinsulin is processed by an enzyme at the position where two basic amino acids (K, R) moieties are present side by side shown by black arrows, yielding insulin and C-peptide (connecting peptide). Most proinsulin was processed while it passes Golgi apparatus to secretory granules, however, some remains without processing and present in secretory granules, and secreted into the blood circulation with insulin. The biopotency of proinsulin is estimated to be about 5-10% of insulin.

When we measure insulin or C-peptide in the blood, in general, we also measure the amount proinsulin because the structure of proinsulin includes those of insulin and C-peptide, if we ignore the three-dimensional structure. So, when we measure insulin by immunological method, we call the assay value IRI (immuno-reactive insulin), discriminating from the real insulin level. IRI includes both insulin and proinsulin levels which reacted with the antibody.

### Amount of proinsulin in IRI

Let's try to estimate the ratio of proinsulin to insulin from data for human blood samples.

The molecular weight of proinsulin is about 9,400, and that of insulin is about 5,800. So, the ratio of molecular weight is 1: 0.62. We will calculate the amount of insulin by weight

as 24U/mg according to international standard preparation.

Normal levels (fasting)

Proinsulin, pmol/l	Reporter
3.3 ~ 10.1 (mean:4.47)	Hampton, 1988
1.5 ~ 27 (mean:14.25)	Naylor, 1987
5.8 ± 3.3	Yoshioka, 1988

If we takes the mean, it ranges 4.5 ~ 14.28 pmol/ml

1 pmol/l of proinsulin is 9400pg/ml = 9.4pg/ml

so 4.5 ~ 14.28 pmol/l = 43 ~ 134 pg/ml

Insulin equivalent from the difference in molecular weight, 27 ~ 83 pg/ml

As normal fasting level of insulin in human subject is about 10 $\mu$ U/ml

= 10/24 ng/ml=420pg/ml. This means 10~30% of IRI may be proinsulin.

### Does ratio of proinsulin to insulin change?

According to clinical reports, the blood proinsulin ratio increases in various cases.

NIDDM ( Type II diabetes mellitus )

In both fasting state and glucose administration, blood levels of proinsulin and the ratio to insulin were reported to be higher in NIDDM patients than in normal subjects. This tendency is more obvious in those patients who show high fasting blood sugar and obesity. Continuous insulin secretion caused by higher blood sugar may results in the secretion of immature secretory granules.

IDDM ( Type I diabetes mellitus )

In the case of untreated subjects, blood insulin levels are low because of impaired biosynthesis of insulin. But in many cases, insulin-treated patients have produced insulin antibody to exogenous insulin, and show higher blood proinsulin levels. This may be due to the binding of proinsulin to insulin antibody which causes the retardation of metabolism of proinsulin. Even some untreated patients show higher proinsulin levels because the presence of antibody.

Obesity

High proinsulin levels are observed in obesity.

As in NIDDM, acceleration of biosynthesis-secretion process by the increase of insulin secretion may cause the release of immature secretory granules.

Insulinoma

In insulinoma patients, hyperproinsulinemia is more obvious than the increase in insulin and C-peptide levels, which is helpful in diagnosis of insulinoma.

Familial hyperproinsulinemia

In hereditary occurring hyperproinsulinemia observed in some families, IRI in secretory granules consisted mostly of proinsulin. This is caused by some abnormality of processing enzyme from proinsulin to insulin, or by possible point mutation in proinsulin gene which produces stable proinsulin against processing enzyme.

## Hyperthyroidism

Increase in gluconeogenesis caused by thyroid hormones results in hyperglycemia, and promote insulin biosynthesis and release, which also increases proinsulin secretion.

As stated above, in most cases, the ratio of proinsulin to insulin may reflect the situation of insulin biosynthetic process.

### Highly specific insulin ELISA kit, S-type

We are providing ordinary assay kits(T, U, UE) and high specific assay kits for insulin(S). S-type has a very low cross-reactivity to proinsulin.

We tried a recovery test by adding proinsulin to assay samples. The assay results are shown below. It proved that proinsulin added to the samples only slightly increased the insulin assay value. This means we can measure insulin precisely in the presence of proinsulin.

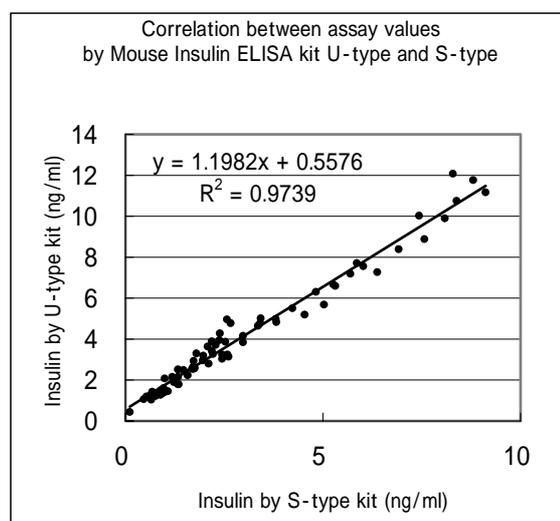
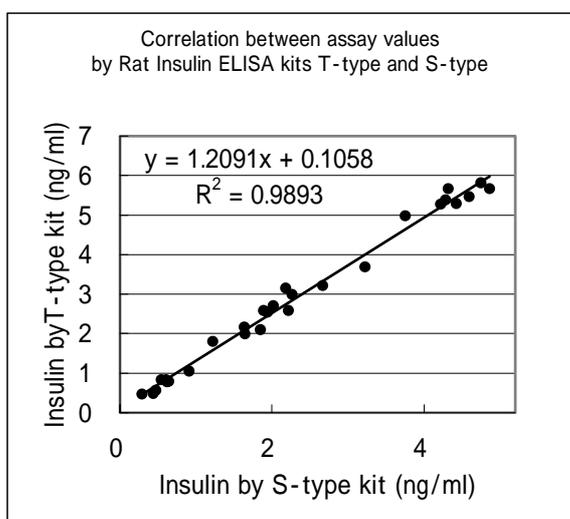
#### Recovery test of proinsulin to rat serum sample n=2

Rat proinsulin added ng/ml	Assay value as insulin ng/ml	Influence assay value / added (%)
0	1.00	-
1.0	1.01	1.0
2.0	1.03	1.5
4.0	1.07	1.8
8.0	1.19	2.4

#### Recovery test of proinsulin to mouse serum sample n=2

Mouse proinsulin added ng/ml	Assay value as insulin ng/ml	Influence assay value / added (%)
0	106.0	-
100	106.9	0.9
500	112.5	1.3
5000	286.0	3.6

Comparison of assay values obtained by ordinary kit (T) and specific kit (S) with rat and mouse serum samples.



As shown in the figures, very high correlation coefficients were obtained with both rat( $R=0.9946$ ) and mouse( $R=0.9868$ ) samples.

The slopes of the regression lines were 1.209 in rat and 1.198 in mouse. This means that type S kits gave about 20% lower assay values than ordinary T-type kits with both rat and mouse samples. This must be caused by improvement of specificity in S-type. Though we should be careful in thinking that the difference is directly related proinsulin, difference of 20% is a reasonable value of proinsulin ratio to IRI in human subjects.

We recommend our customers to use our highly specific insulin kit S-type in measurements of rat and mouse insulin.

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