Rat C-peptide and proinsulin

Rat C-peptide is a polypeptide molecule comprising 31 amino acid residues with molecular mass of about 3.2 kDa. C-peptide is originated from proinsulin, which is synthesized in the β-cells of the Islets of Langerhans and cleaved enzymatically releasing insulin and C-peptide. Scheme of proinsulin processing is represented on Fig. 1. Proinsulin is cleaved by two Ca-dependent endopeptidases (prohormone convertases) PC2 and PC3. Endopeptidase PC2 (type II) cleaves at the A/C chain junction of proinsulin (between amino acid residues 65 and 66) and PC3 (type I) cleaves at the B/C junction (between amino acid residues 32 and 33)(1). Carboxypeptidase H removes basic amino acids from the C-terminus of proinsulin-derived peptides to generate insulin and C-peptide(2). In contrast to PC3 that recognizes des-64,65 proinsulin and intact proinsulin as similar substrates, PC2 has a stronger preference for des-31,32 proinsulin compared to intact proinsulin. This mechanism provides the preferential route of proinsulin conversion via des-31,32-proinsulin (type I processing) (Fig. 1: Ia and Ib)(3). Des- rather than split- forms prevail in the blood and the major circulating form of partially processed proinsulin is des-31,32 proinsulin (Fig. 1: Ib). The term “proinsulin” refers to non-processed or “intact proinsulin” whereas term “partially processed proinsulin” is used for split- and des- forms of proinsulin molecule (Fig. 1: Ia, Ib, IIa and IIb).

Insulin, one of the two products of proinsulin processing, regulates carbohydrate metabolism. Insulin has a highly conservative sequence over mammals, reptiles, birds and fish. On the contrary, C-peptide (physiological activity was not shown) demonstrates considerable interspecies variability. For the most of species only one form of proinsulin is described. Unlike others, rats and mice produce two proinsulin isoforms – I and II, which differ from each other in two (rat) or three (mouse) amino acid residues of the C-peptide part of proinsulin (Table 1).

Analysis of proinsulin synthesis and processing, insulin and C-peptide clearance are very important for better understanding of carbohydrate metabolism abnormalities. Assays for insulin, proinsulin and C-peptide are widely used for monitoring of hypoglycemia, pathogenesis and treatment of diabetes mellitus. It was demonstrated that C-peptide measurements in blood or urine have several advantages over the direct insulin quantification. C-peptide measurements could be the only method to determine insulin production in case of diabetes treatment when endogenous insulin is mixed in blood with the exogenous molecule. Being released into the bloodstream insulin is utilized very fast by liver. Fast excretion and fast elimination results in considerable fluctuations of insulin concentrations in the blood. C-peptide is eliminated and degraded mainly by kidneys and this process is not so impetuous as insulin elimination. Also insulin in blood is less stable than C-peptide. As a result the half life of insulin in blood is significantly shorter (4 min) than that of C-peptide (33 min)(4). Finally, hemolysis is known to reduce significantly measured insulin concentration(5). Consequently C-peptide seems to be more reliable indicator of insulin production than insulin by itself.

HyTest offers monoclonal antibodies specific to different parts of rat C-peptides I and II. Thoroughly chosen epitopes and original approaches for selecting specific monoclonal antibodies allowed us to develop highly sensitive and specific antibodies which make C-peptide detection possible without cross-reactivity with native proinsulin or some forms of partially processed proinsulin. **Moreover we also offer pairs of antibodies that are able to detect either both isoforms of rat C-peptide (C-peptides I and II) or one of two isoforms (C-peptide I or II).** Finally, we have generated monoclonal antibodies that specifically detect intact and partially processed proinsulin and do not interact with free C-peptide.

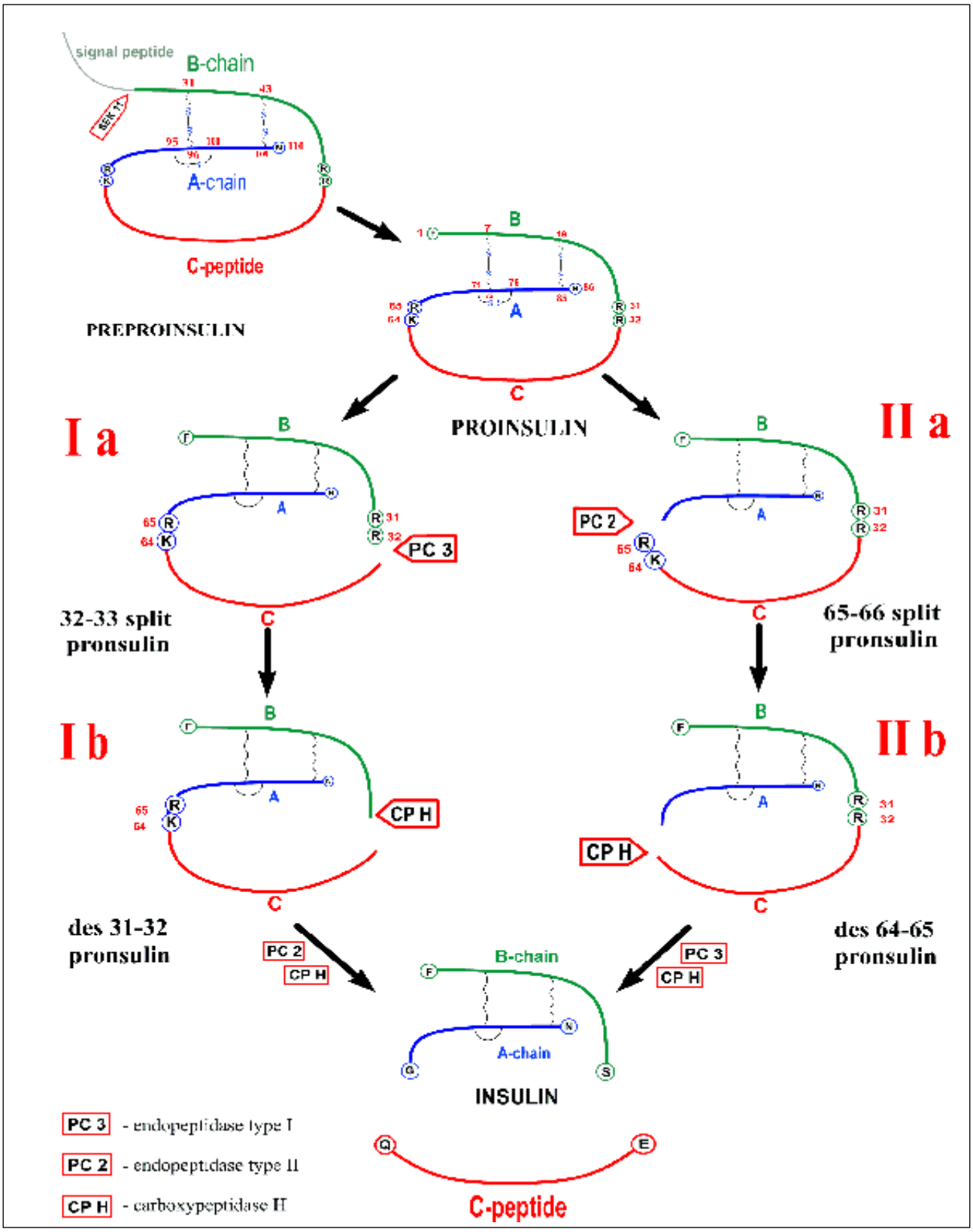

Figure 1. Processing of proinsulin.

Table 1. Amino-acid sequence of C-peptides. Differences in sequence are marked with red.

Peptide	1																												31
rat CI	E	V	E	D	P	Q	V	P	Q	L	E	L	G	G	G	P	E	A	G	D	L	Q	T	L	A	L	E	V	A	R	Q
rat CII	E	V	E	D	P	Q	V	A	Q	L	E	L	G	G	G	P	G	A	G	D	L	Q	T	L	A	L	E	V	A	R	Q
mouse CI	E	V	E	D	P	Q	V	E	Q	L	E	L	G	G	S	P	G	-	-	D	L	Q	T	L	A	L	E	V	A	R	Q
mouse CII	E	V	E	D	P	Q	V	A	Q	L	E	L	G	G	G	P	G	A	G	D	L	Q	T	L	A	L	E	V	A	Q	Q

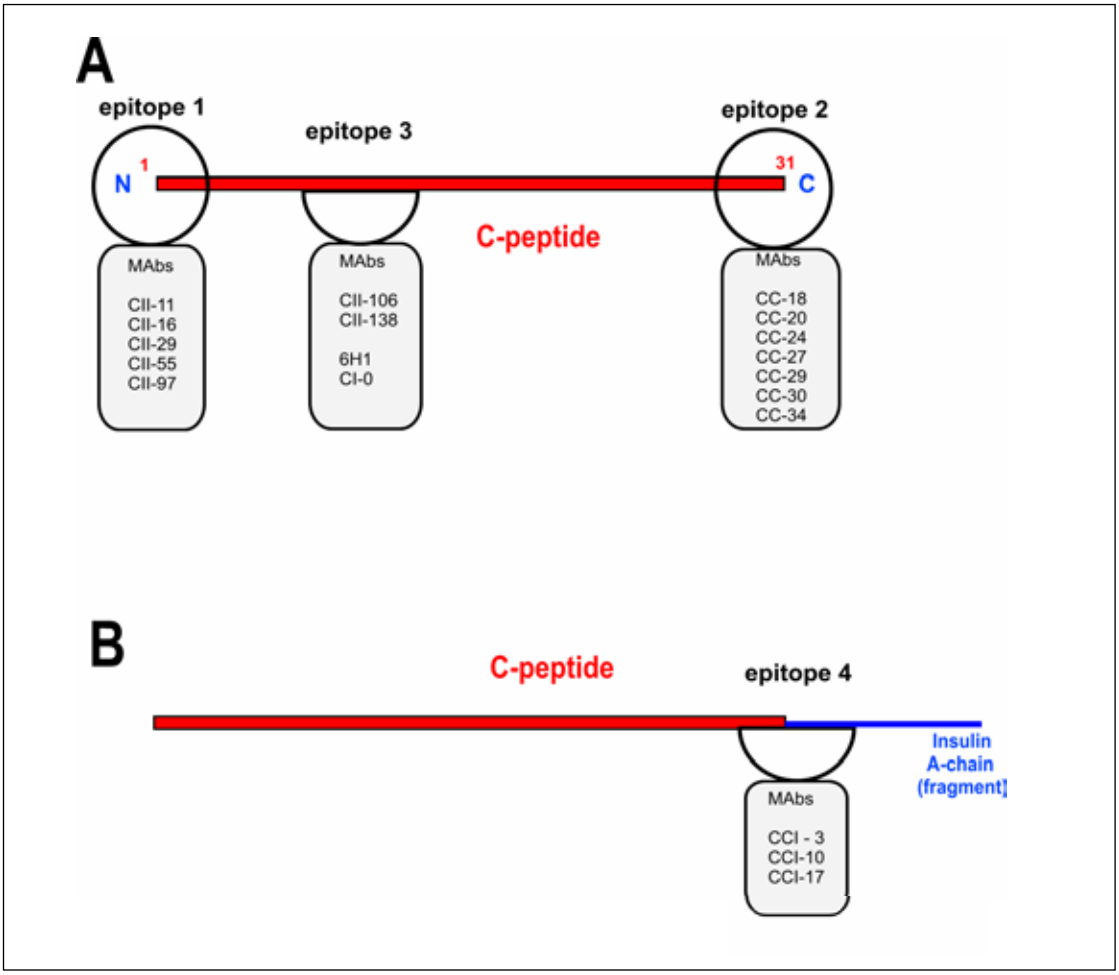

Figure 2. Epitope specificity of anti-rat C-peptide antibodies.

1. Anti-rat C-peptide monoclonal antibodies

Host animal:	*Mice Balb/c*
Cell line used for fusion:	*Sp2/0*
Antigen:	*Rat C-peptides I and II fragments conjugated with a carrier protein*
Specificity:	*Specific to rat and mouse C-peptides*
Purification method:	*Protein A affinity chromatography*
Presentation:	*MAb solution in PBS with 0.1% sodium azide*

Hybridoma clones have been derived from hybridization of Sp2/0 myeloma cells with the spleen cells of Balb/c mice immunized with fragments of rat C-peptide conjugated with a carrier protein.

HyTest offers MAbs specific to different epitopes of rat C-peptide molecule. The epitope specificity of anti-rat C-peptide antibodies is shown on Fig. 2.

All antibodies, which recognize the terminal parts of C-peptide molecule (N-terminus: epitopes 1 and C-terminus: epitope 2, Fig. 1), have no cross-reaction with proinsulin (Fig. 3) and could be used for the development of C-peptide immunoassays. These antibodies recognize both isoforms of rat C-peptide (I and II) with the same affinity.

As it was already mentioned above, two isoforms of rat C-peptide, C-peptide I and C-peptide II, are described in literature. We have developed monoclonal antibodies which:
1: recognize only C-peptide I
2: recognize only C-peptide II
3: recognize both forms with almost equal efficiency (see Table 2 for MAb specificity).

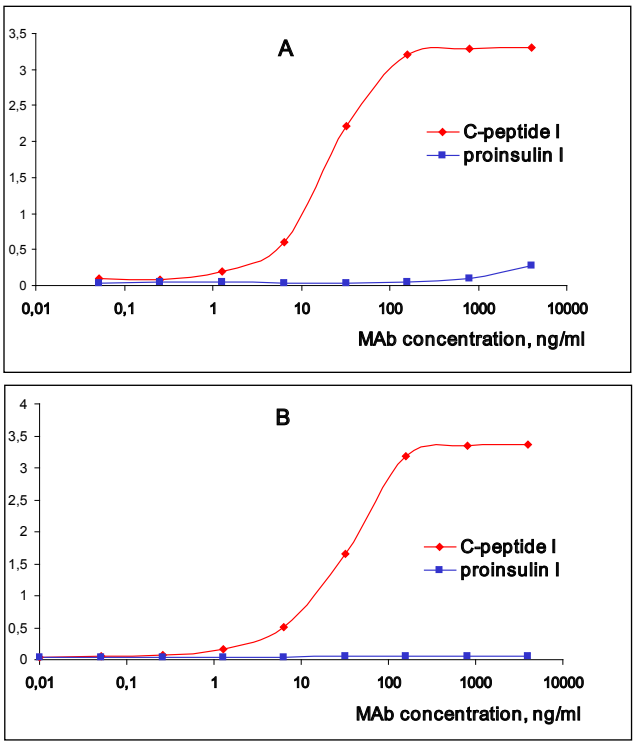

Figure 3. Titration curves of MAbs specific to epitopes 1 and 2 of rat C-peptide.

A. MAb CII-16 (epitope 1)
Antigens:
- *N-terminal part of rat C-peptide I conjugated with the carrier protein: 0.04 µg/well*
- *rat proinsulin I: 0.04 µg/well*

B. MAb CC34 (epitope 2)
Antigens:
- *rat C-peptide I: 0.01 µg/well*
- *rat proinsulin I: 0.04 µg/well*

Titration curves of monoclonal antibodies with different specificity to rat C-peptide isoforms are shown in Fig. 4.

Besides immunodetection of rat C-peptide our antibodies could be also used for mouse C-peptide immunodetection. Because of similar amino acid sequences of rat and mouse C-peptides big part of monoclonal antibodies that are specific to rat C-peptides recognize also mouse C-peptides with the same affinity. Specificity of all antibodies to mouse C-peptides I and II is presented in Table 2.

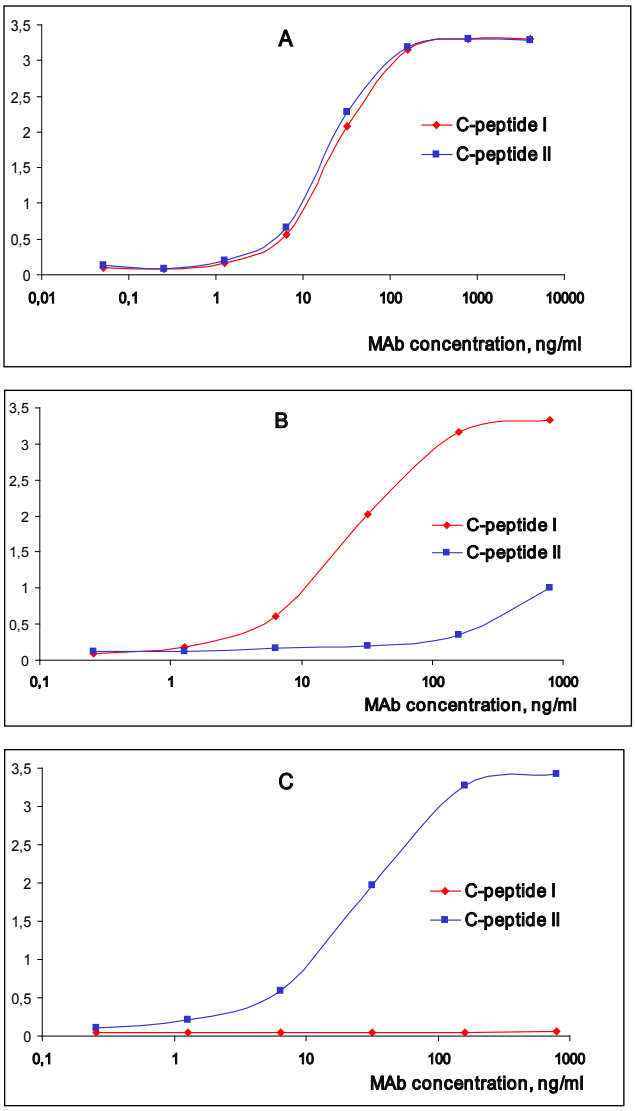

Figure 4. Titration curves of monoclonal antibodies specific to different isoforms of rat C-peptide.

A. Titration curve for MAb CII-29 (detects both C-peptides I and II)

B. Titration curve for MAb 6H1 (C-peptide I specific)

C. Titration curve for MAb CII-106 (C-peptide II specific)

Antigens:

- *N-terminal part of rat C-peptide I conjugated with the carrier protein: 0.05 µg/well*
- *N-terminal part of rat C-peptide II conjugated with the carrier protein: 0.05 µg/well*

Table 2. Cross-reaction of rat C-peptide specific antibodies with rat and mouse C-peptides I and II and rat proinsulin in direct ELISA

MAbs	Rat C-peptide I	Rat C-peptide II	Mouse C-peptide I	Mouse C-peptide II	Rat proinsulin
CII-11, CII-29	+++	+++	+++	+++	+
CII-55, CII-97	+++	+++	+++	+++	++
CII-16	+++	+++	+++	+++	low
6H1, CI-0	++	low	-	low	++
CII-106, CII-138	-	+++	low	+++	not tested
CC18, CC20, CC24, CC27, CC29, CC30, CC34	+++	+++	+++	+	-

1.1. Antibody applications

1.1.1. Rat C-peptide quantitative immunoassay

HyTest recommends several pairs of monoclonal antibodies that could be used for the development of rat C-peptide immunoassays. For precise rat C-peptide immunodetection both antibodies utilized in sandwich immunoassay should specifically interact only with free C-peptide and have no cross-reaction with proinsulin. As it was mentioned above, such antibodies recognize either N-terminal part of C-peptide molecule (epitope 1) or the C-terminus of C-peptide (epitope 2). Sandwich immunoassays utilizing such antibodies recognize rat C-peptides with high sensitivity and do not recognize proinsulin.

Calibration curve for the immunoassay with monoclonal antibody CII-29 used for capture and monoclonal antibody CC24 used for detection is presented in Fig. 5.

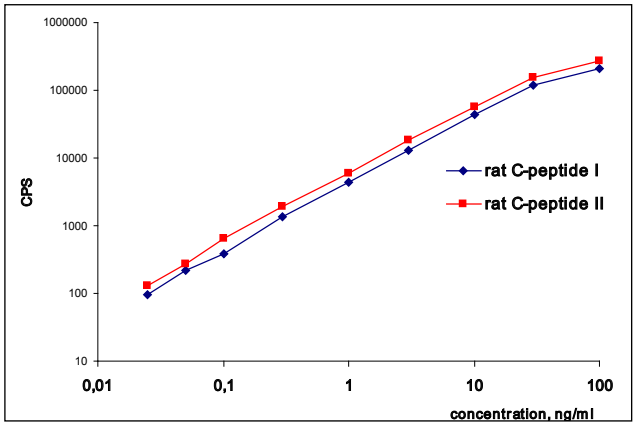

Figure 5. Calibration curve for one step rat C-peptide immunoassay.
Capture MAb (biotinylated): CC24, 0.4 µg/well
Detection MAb (Eu-labeled): CII-29, 0.4 µg/well
Streptavidin coated plates
Incubation time: 30 min

Recommended pairs for sandwich immunoassays (capture - detection):
CC24 – CII-29
CII-29 – CC24
CII-55 – CC24
CII-97 – CC24
CC34 – CII-11
CC27 – CII-29

Cross-reactivity for all recommended pairs with native rat proinsulin is less than 0.1%.

1.1.2. Immunoassays for separate rat C-peptide I or C-peptide II immunodetection

HyTest offers pairs of monoclonal antibodies that could be utilized in sandwich immunoassays for separate rat C-peptide I or C-peptide II immunodetection. In such two-site MAb combinations one antibody is specific to epitope 2 (recognizes both forms of the antigen), whereas second monoclonal antibody is either C-peptide I or C-peptide II -specific (Fig. 6). However using such assay for C-peptide isoforms measurements the contribution of des-31,32 form (partially processed proinsulin (Fig. 1) should be considered.

The best two-site MAb combinations for rat C-peptide I -specific sandwich immunoassay:
CC18 – 6H1
CC34 – 6H1
CC34 – CI-0

The best two-site MAb combinations for rat C-peptide II -specific sandwich immunoassay:
CC18 – CII-106
CC34 – CII-106
CC34 – CII-138

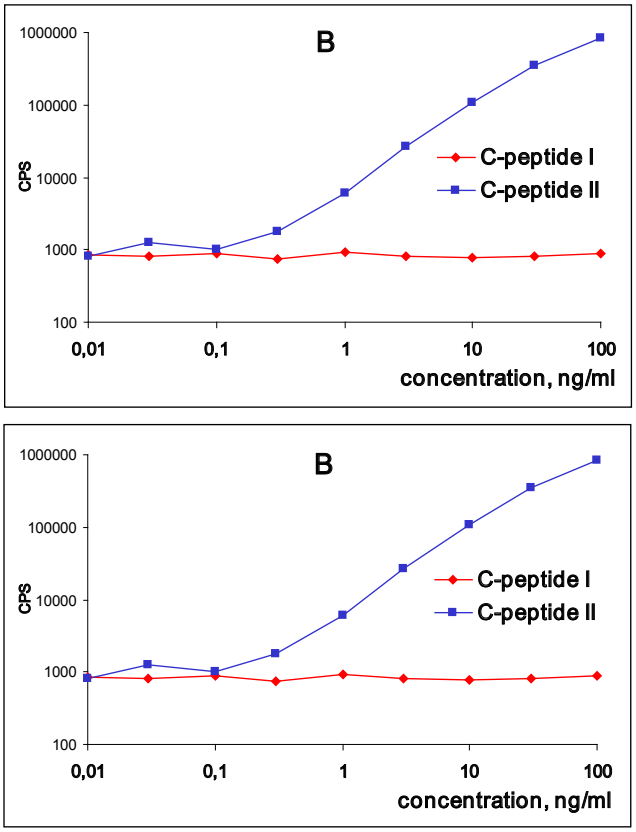

Figure 6. Calibration curve for separate rat C-peptide I and C-peptide II immunodetection in sandwich immunoassay.

A. Rat C-peptide I immunodetection.
Capture MAb: CC18, 1 µg/well
Detection MAb (Eu-labeled): 6H1, 0.2 µg/well
Incubation time: 30 min
B. Rat C-peptide II immunodetection.
Capture MAb: CC34, 1 µg/well
Detection MAb (Eu-labeled): CII-106: 0.2 µg/well
Incubation time: 30 min

2. Anti-rat proinsulin monoclonal antibodies

Host animal:	*Mice Balb/c*
Cell line used for fusion:	*Sp2/0*
Antigen:	*Rat proinsulin fragments conjugated with a carrier protein*
Specificity:	*Specific to rat proinsulin*
Purification method:	*Protein-A affinity chromatography*
Presentation:	*MAb solution in PBS with 0.1% sodium azide*

HyTest offers monoclonal antibodies that recognize proinsulin with high affinity and do not have cross-reaction with rat C-peptide. The epitope of such antibodies includes the site of proinsulin cleavage by PC2 (approximately residues 62-70 of proinsulin; Fig. 2B). For rat proinsulin immunodetection we recommend to use such antibodies in pairs with antibodies specific to epitope 3 (Fig. 2). However it is necessary to consider that such assay should also recognize partially processed proinsulin (32, 33 split and des-31,32 forms, Fig. 1) and the presence of these peptides in the sample could influence proinsulin measurements.

Calibration curve for rat proinsulin I sandwich immunoassay is presented in Fig. 7.

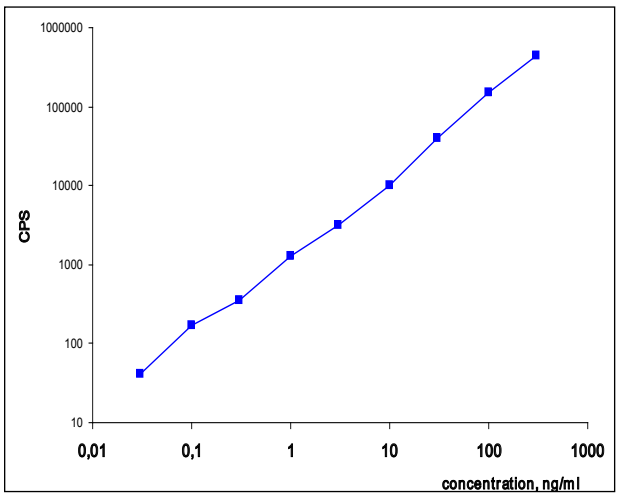

Figure 7. Calibration curve for rat proinsulin I immunodetection in one step sandwich immunoassay.
Capture MAb (biotinylated): CCI-17, 0.2 µg/well
Detection MAb (Eu-labeled, Cat.# 2I3): CI-0: 0.2 µg/well
Incubation time: 30 min

Best pairs for sandwich immunoassays (coating-detection):

Proinsulin I and II immunodetection:
CCI-17 – CII-55 (Cat.# 2I3)
CCI-17 – CII-97 (Cat.# 2I3)

Proinsulin I immunodetection:
CCI-17 – CI-0 (Cat.# 2I3)
CCI-17 – 6H1 (Cat.# 2I3)

Ordering inforamation:

MAb	Cat. #	Specificity	Subclass	Applications
CC18	2I3	rat C-peptides I and II, mouse C-peptide I	IgG1	EIA, Sandwich immunoassay (coating)
CC20	2I3	rat C-peptides I and II, mouse C-peptide I	IgG1	EIA, Sandwich immunoassay (coating)
CC24	2I3	rat C-peptides I and II, mouse C-peptide I	IgG1	EIA, Sandwich immunoassay (conjugate)
CC27	2I3	rat C-peptides I and II, mouse C-peptide I	IgG1	EIA, Sandwich immunoassay (conjugate)
CC29	2I3	rat C-peptides I and II, mouse C-peptide I	IgG1	EIA, Sandwich immunoassay (coating)
CC30	2I3	rat C-peptides I and II, mouse C-peptide I	IgG1	EIA, Sandwich immunoassay (conjugate)
CC34	2I3	rat C-peptides I and II, mouse C-peptide I	IgG1	EIA, Sandwich immunoassay (coating)
CII-11	2I3	rat C-peptides I and II, mouse C-peptides I and II	IgG1	EIA, Sandwich immunoassay (coating, conjugate)
CII-16	2I3	rat C-peptides I and II, mouse C-peptides I and II	IgG1	EIA, Sandwich immunoassay (conjugate)
CII-29	2I3	rat C-peptides I and II, mouse C-peptides I and II	IgG1	EIA, Sandwich immunoassay (coating, conjugate)
CII-55	2I3	rat C-peptides I and II, mouse C-peptides I and II	IgG1	EIA, Sandwich immunoassay (coating)
CII-97	2I3	rat C-peptides I and II, mouse C-peptides I and II	IgG1	EIA, Sandwich immunoassay (coating)
CII-106	2I3	rat C-peptide II, mouse C-peptide II	IgG1	EIA, Sandwich immunoassay (conjugate)
CII-138	2I3	rat C-peptide II, mouse C-peptide II	IgG1	EIA, Sandwich immunoassay (conjugate)
CI-0	2I3	rat C-peptide I	IgG1	EIA, Sandwich immunoassay (conjugate)
6H1	2I3	rat C-peptide I	IgG1	EIA, Sandwich immunoassay (conjugate)

Ordering inforamation:

MAb	Cat. #	Specificity	Subclass	Applications
CCI-3	2PR8	rat proinsulin	IgG2b	EIA, Sandwich immunoassay (coating)
CCI-10	2PR8	rat proinsulin	IgG1	EIA, Sandwich immunoassay (coating)
CCI-17	2PR8	rat proinsulin	IgG1	EIA, Sandwich immunoassay (coating)

MAbs were tested on their ability to recognize rat proinsulin I. This part of proinsulin molecule has the same sequence in rat proinsulin II and mouse proinsulin I. So we can assume that these antibodies would also recognize rat proinsulin II and mouse proinsulin I with the same efficiency as rat proinsulin I (not tested).

References:

1. *Davidson HW, et al. (1988) Intraorganellar calcium and pH control proinsulin cleavage in the pancreatic beta cell via two distinct site-specific endopeptidases. Nature 333(6168), 93-96.*
2. *Davidson HW and Hutton JC (1987) The insulin-secretory-granule carboxypeptidase H. Purification and demonstration of involvement in proinsulin processing. Biochem J 245(2), 575-582.*
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