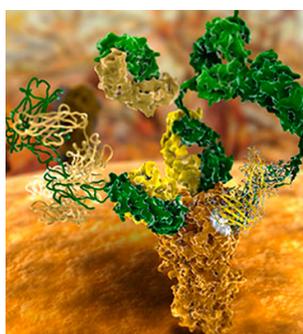


Rat C-peptide and proinsulin



Rat C-peptide is a polypeptide molecule comprising 31 amino acid residues with a 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. A 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 (physi-

CLINICAL UTILITY

- Diabetes mellitus
- Hypoglycemia

ological activity was not shown) demonstrates considerable interspecies variability. For most 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).

For analysis of proinsulin synthesis and processing, insulin and C-peptide clearance are very important for a 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 the 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. 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.

Advanced ImmunoChemical 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 led to the development of highly sensitive and specific antibodies which make C-peptide detection possible without cross-reactivity with native proinsulin or some forms of partially processed pro-

insulin. **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 provide monoclonal antibodies that specifically detect intact and partially processed proinsulin and do not interact with free C-peptide.

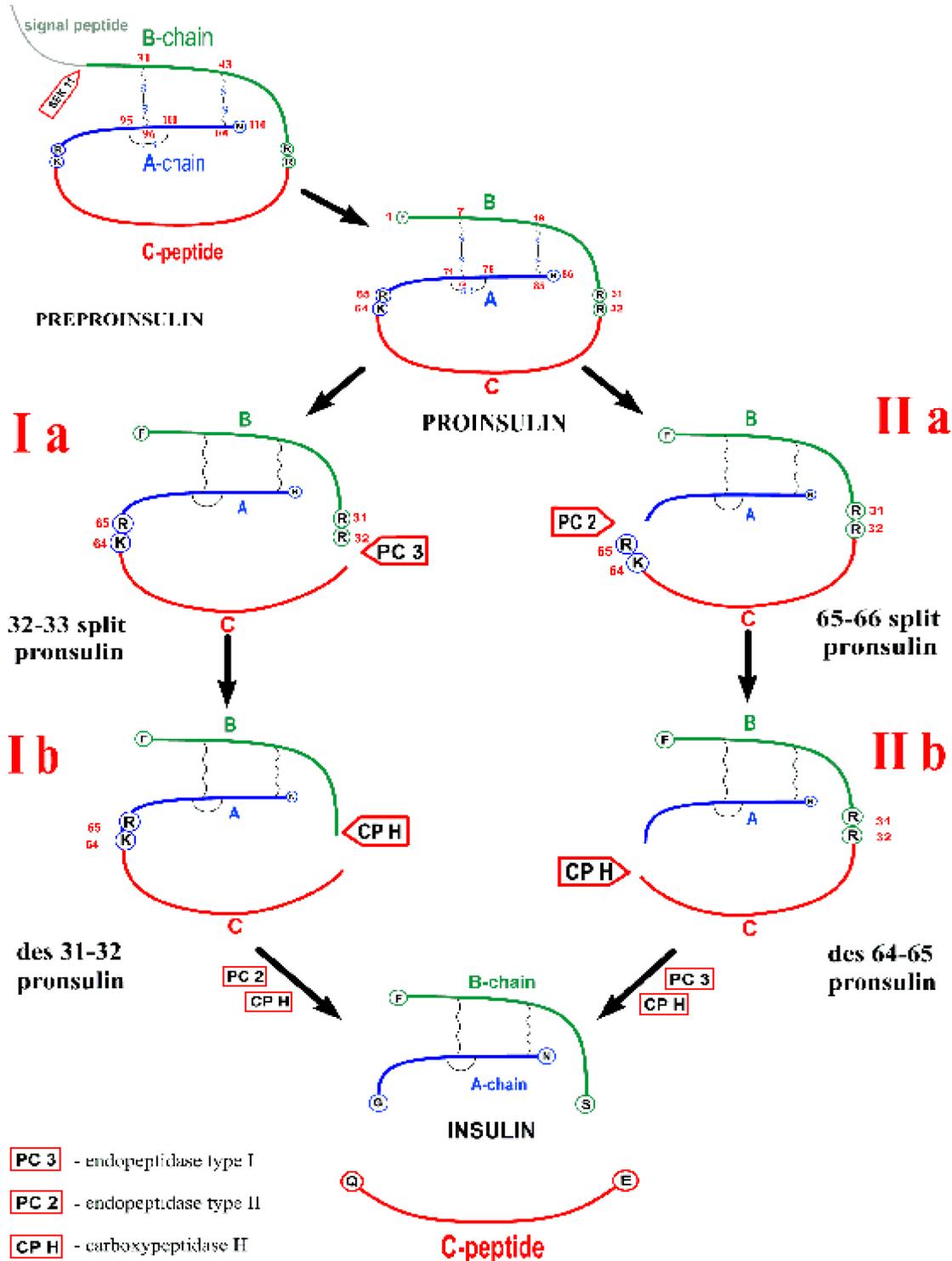


Figure 1. Processing of proinsulin

2 AssayNotes | Rat C-peptide and 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	

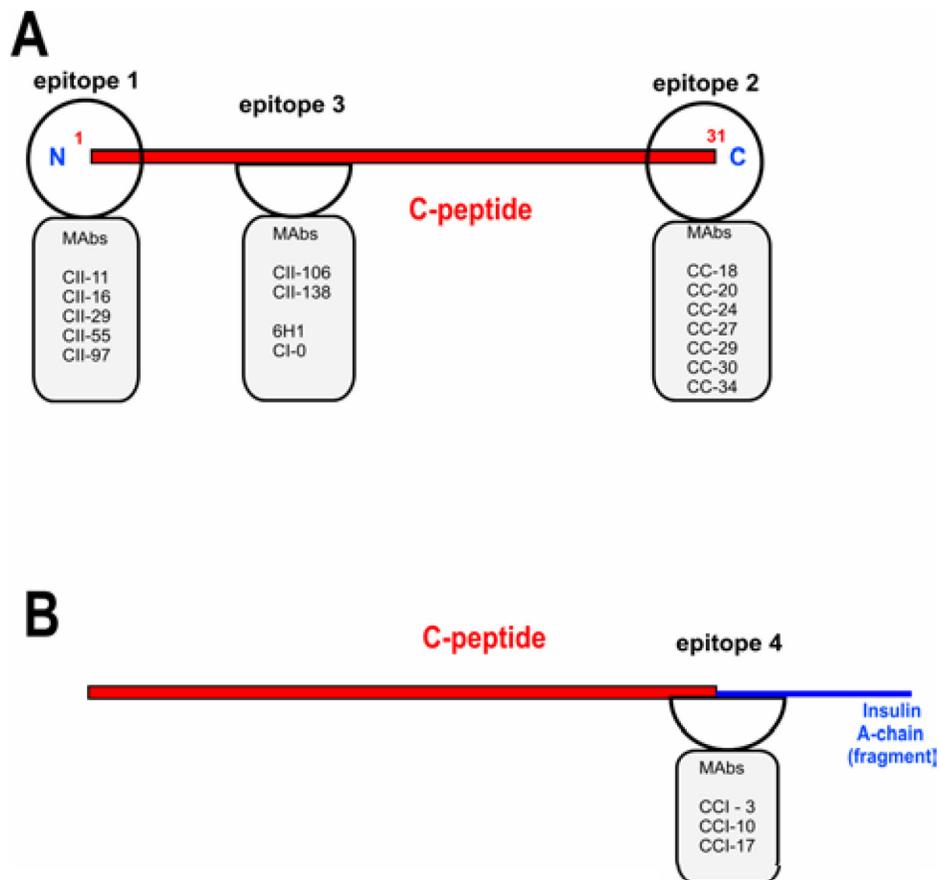


Figure 2. Epitope specificity of anti-rat C-peptide antibodies.

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.

Advanced ImmunoChemical 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. Our monoclonal antibodies:

- 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).

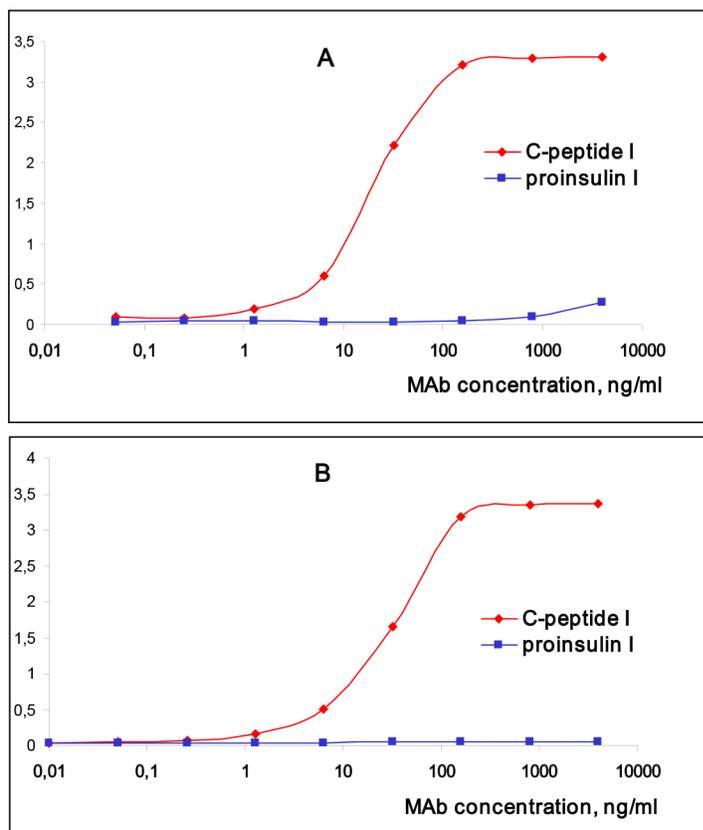


Figure 3. Titration curves of MABs specific to epitopes 1 and 2 of rat C-peptide.

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 antibodies can be also used for mouse C-peptide immunodetection. Because of similar amino acid sequences of rat and mouse C-peptides it is important that 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.

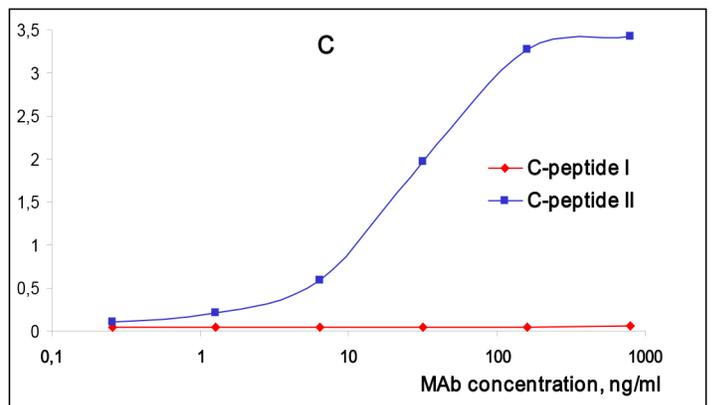
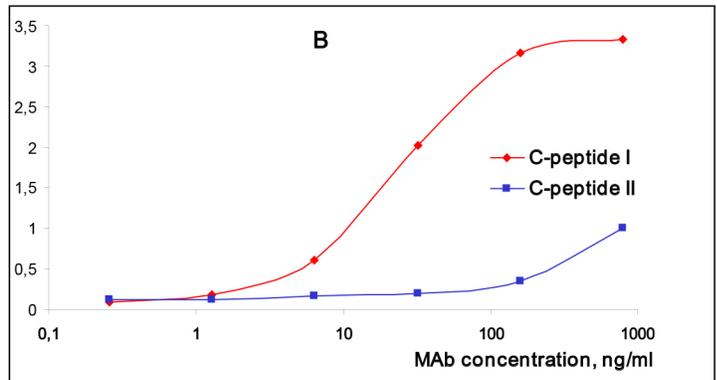
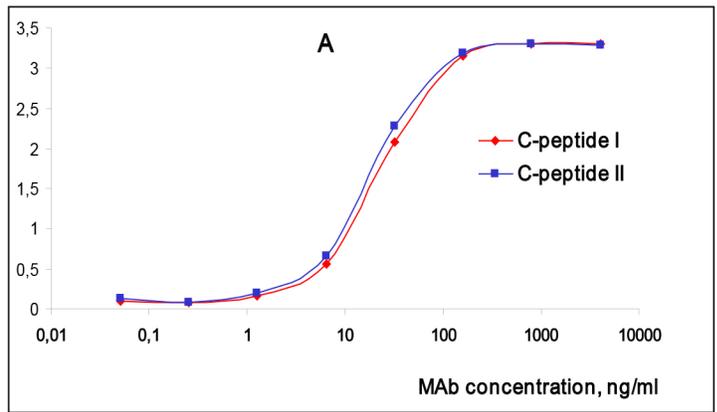


Figure 4. Titration curves of monoclonal antibodies specific to different isoforms of rat C-peptide.

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

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	+++	+++	+++	+	-

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

2. Anti-rat proinsulin monoclonal antibodies

1.1.1 Rat C-peptide quantitative immunoassay

Several pairs of monoclonal antibodies can 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.

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%.

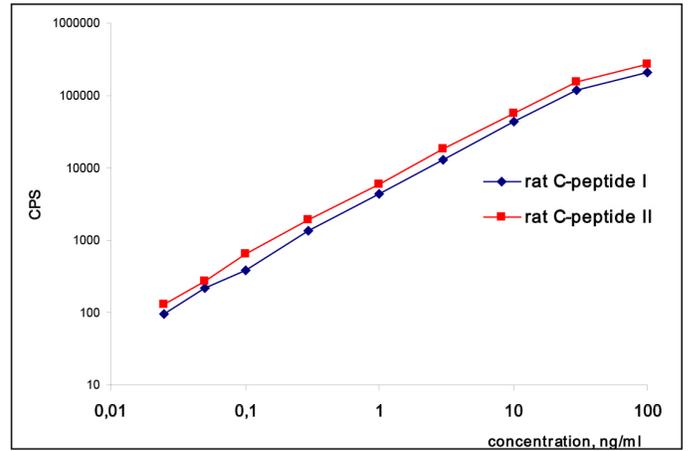


Figure 5. Calibration curve for one step rat C-peptide immunoassay.
Capture MAb (biotinylated): CC24, 0.4 µg/well Detection MAb (Eu-labeled): CII-29, 0.4 µg/well Streptavidin coated plates
Incubation time: 30 min

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

Advanced ImmunoChemical offers pairs of monoclonal antibodies that can 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

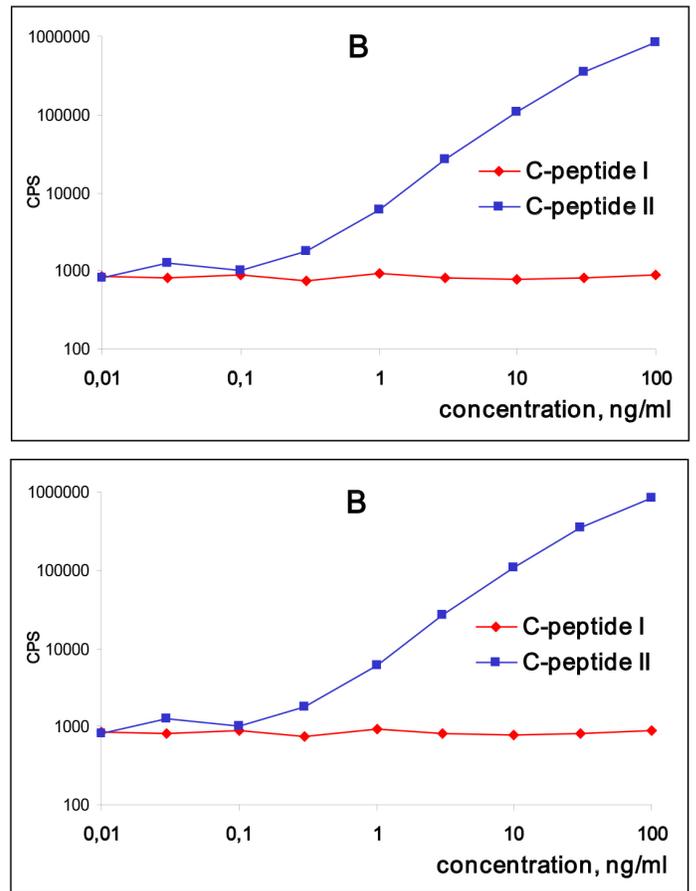


Figure 6. Calibration curve for separate rat C-peptide I and C-peptide II immunodetection in sandwich immunoassay.

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

Advanced ImmunoChemical offers monoclonal antibodies that recognize proinsulin with high affinity and do not cross-react 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 it is recommended 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.

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)

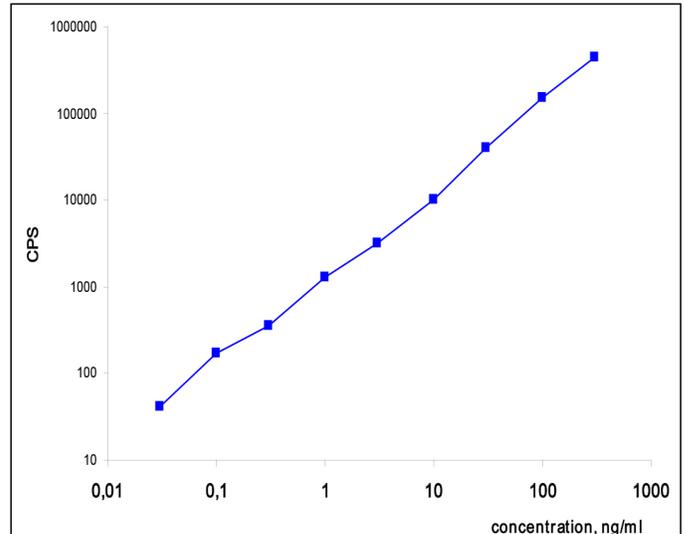


Figure 7. Calibration curve for rat proinsulin I immunodetection in one step sandwich immunoassay.

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

Ordering Information:

MAb	Cat #	Specificity	Subclass	Applications
CC24	1-CP-r	rat C-peptides I and II, mouse C-peptide I	IgG1	EIA, Sandwich immunoassays (conjugate)
CC27	1-CP-r	rat C-peptides I and II, mouse C-peptide I	IgG1	EIA, Sandwich immunoassays (conjugate)
CC34	1-CP-r	rat C-peptides I and II, mouse C-peptide I	IgG1	EIA, Sandwich immunoassays (coating)
CII-11	1-CP-r	rat C-peptides I and II, mouse C-peptides I and II	IgG1	EIA, Sandwich immunoassays (coating, conjugate)
CII-29	1-CP-r	rat C-peptides I and II, mouse C-peptides I and II	IgG1	EIA, Sandwich immunoassays (coating, conjugate)
CII-55	1-CP-r	rat C-peptides I and II, mouse C-peptides I and II	IgG1	EIA, Sandwich immunoassays (coating)

Ordering Information:

MAb	Cat #	Specificity	Subclass	Applications
CCI-17	1-PI-r	rat proinsulin	IgG1	EIA, Sandwich immunoassays (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. It is assumed that these antibodies would also recognize rat proinsulin II and mouse proinsulin I with the same efficiency as rat proinsulin I (not tested).

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