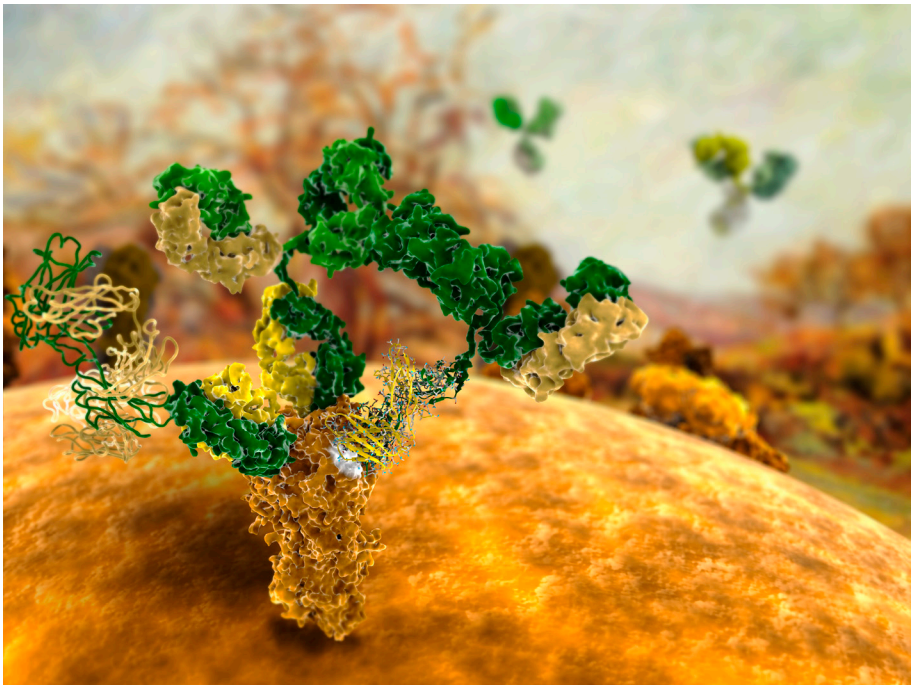
Advanced ImmunoChemical Inc.
Providing for the Advancement of Medical Science

Metabolic Syndrome
Antibodies and Antigens



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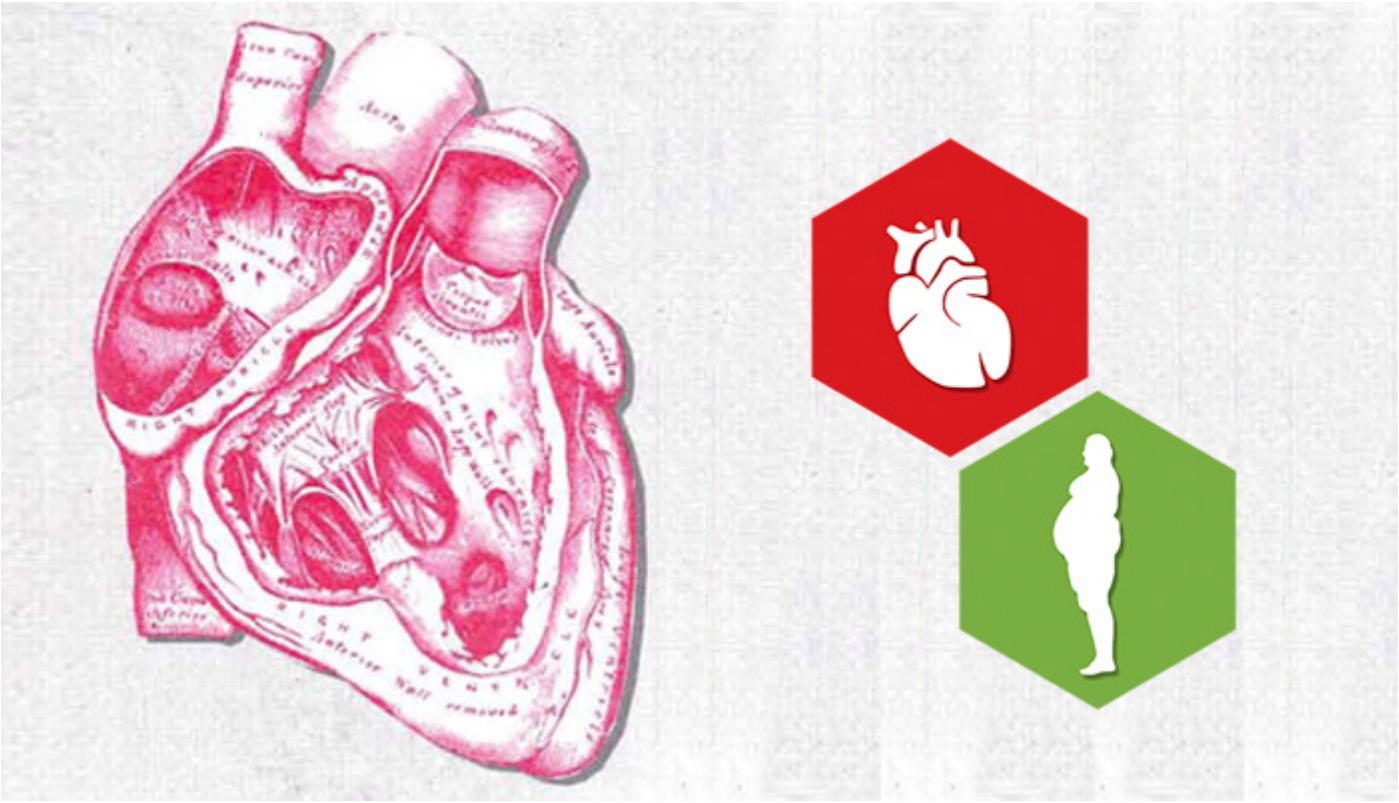
Introduction

Metabolic syndrome is a cluster of conditions that increases the likelihood of cardiovascular heart diseases and diabetes. These metabolic risk factors include abdominal obesity, elevated blood pressure, insulin resistance, high blood sugar levels and abnormal blood cholesterol levels. Both genetic factors and the lifestyle of the individual, including limited physical activity and excess weight, are considered to be underlying causes of metabolic syndrome.

Estimates by the American Heart Association suggest that 20-25% of US adults have metabolic syndrome. It should be noted that there is currently no unified definition of metabolic syndrome. Instead, several organizations including the World Health Organization (WHO), the International Diabetes Federation (IDF) and the European Group for the study of Insulin Resistance (EGIR), have presented their own individual criteria for metabolic syndrome. These criterions have common features but also differing parameters and this inevitably complicates the comparisons of different studies. Nevertheless, as obesity rates grow it is expected to also result in an increase in the incidence of metabolic syndrome. At Advanced ImmunoChemical, we provide immunological reagents — antibodies and antigens — that enable the development of quantitative immunoassays for the detection of various biomarkers, such as adiponectin, insulin and glycated hemoglobin.

Please note that in this brochure the monoclonal antibodies (MAbs) are only listed according to the analyte that they recognize. In most cases there are several different MAbs available under one catalogue number. More detailed information regarding the performance of our products, a full list of individual MAbs and recommendations for capture-detection antibody pairs (when available) can be found on our website at www.advimmuno.com.



Courtesy of HyTest, Ltd

Adiponectin

Adiponectin is an abundant protein hormone that belongs to a family of adipokines. It is expressed mostly by adipocytes and is an important regulator of lipid and glucose metabolism. Adiponectin is an insulin- sensitizing hormone with anti-diabetic, anti-inflammatory and anti-atherogenic properties (1). It has been shown that the amount of adiponectin in blood reduces in cases of patients suffering from Type 2 diabetes mellitus or coronary artery diseases, or who are insulin-resistant (2-6).

Human adiponectin consists of 244 amino acid residues and has a distinct domain structure: It contains both collagen-like and globular C1q-like domains. Collagen-like parts of three adiponectin molecules can form a triple coiled coil structure very similar to that in collagen (7). C1q-like domains form a “head” of adiponectin globula (Figure 1) and share a considerable degree of structural similarity to complement component C1q.

In blood, adiponectin is found as trimers (low- molecular weight form, LMW), hexamers (medium molecular weight form, MMW) and higher order multimers (high molecular weight form, HMW). The exact structure of the HMW form of adiponectin is not yet known. Most likely several combined hexamers and/or trimers form the high-molecular weight form of adiponectin. It has been suggested that different oligomeric forms exist in blood as separate moieties and do not convert into one another (8). Adiponectin oligomers are capable of binding Ca2+ ions, which are thought to participate in the maintenance of conformational stability of adiponectin (9).

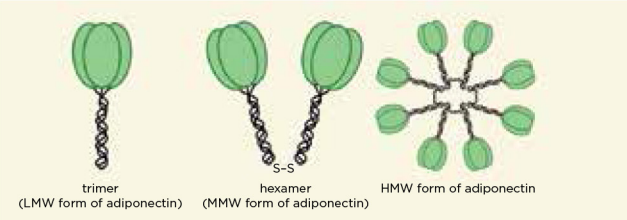


Figure 1. Schematic representation of the oligomeric forms of adiponectin.

CLINICAL UTILITY

- **Type 2 diabetes**

The concentration of total adiponectin in the blood is approximately 3-30 µg/ml, whereas the concentration of the closest structural homolog of adiponectin, C1q, is approximately 80-200 µg/ml. Therefore, it is critical that anti-adiponectin antibodies do not cross-react with human C1q (10). Some authors describe significant gender differences in adiponectin level in healthy adults. These differences could contribute to discrepancies in adiponectin concentrations reported by various authors. It has been suggested that the concentration of the HMW form of adiponectin or HMW/total adiponectin ratio correlates with insulin resistance and metabolic syndrome better than just the concentration of total adiponectin (11-12).

Reagents for the development of a reliable adiponectin assay

Advanced ImmunoChemical offers several anti-human adiponectin monoclonal antibodies and a native purified adiponectin that enable the development of adiponectin specific immunoassays.

Monoclonal antibodies specific to human adiponectin

Hybridoma clones were derived from hybridization of Sp2/0 myeloma cells with spleen cells of Balb/c mice immunized with either human recombinant adiponectin or native human adiponectin.

All antibodies were tested in direct ELISA for cross-reaction with C1q, which is the most abundant adiponectin homolog in blood. None of the selected MAbs showed any cross-reaction with human C1q.

Sandwich immunoassay

All MAbs were tested in two-site combinations as capture or detection antibodies in sandwich ELISA with native adiponectin. Seven two-site combinations were selected for the development of sandwich immunoassays on the basis of sensitivity and specificity to different oligomeric forms of adiponectin:

Adn20 - Adn23

Adn36 - Adn27

Adn94 - Adn63

Adn279 - Adn94

Adn214 - Adn27

Adn222 - Adn94

Adn305 - Adn279

A representative curve demonstrating detection of purified native adiponectin by the assay Adn279-Adn94 is shown on Fig. 2.

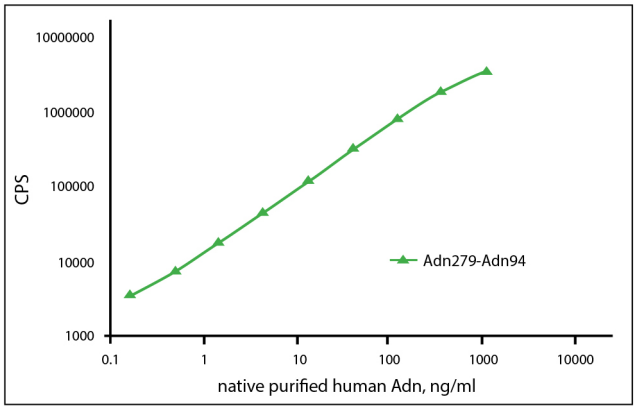


FIGURE 2. Calibration curve for sandwich adiponectin immunoassay.
MAb Adn279 was used as a coating (1 µg/well), MAb Adn94 was labeled with stable Eu^{3+} chelate and was used as a detection (0.2 µg/well) antibody. Native adiponectin purified from human plasma was used as a calibrator

All assays were tested with serial dilutions of normal human serum to evaluate the interaction of MAbs with native adiponectin in a complex environment. All assays demonstrated a steady decrease of signal correlating with the degree of serum dilution. The representative titration curve for the assay Adn94-Adn63 (capture antibody-detection antibody, respectively) is shown in Fig. 3.

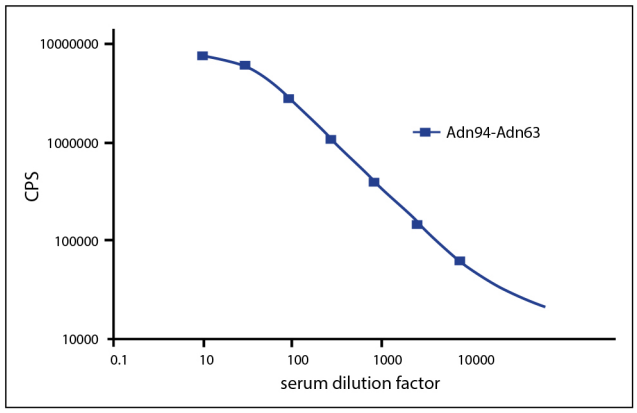


FIGURE 3. Normal human serum titration curve in sandwich immunofluorescent assay. *Adn94 MAb was used as a coating antibody (1 µg/well), MAb Adn63 was used as a detection antibody (0.2 µg/well). Normal human serum, serially diluted with phosphate- buffered saline (10 mM K-phosphate, pH 7.4, 150 mM NaCl, 0.1% Tween-20) was used as an antigen.*

Assays Adn36-Adn27 and Adn20-Adn23 react differently with adiponectin in serum and citrate plasma (Fig. 4). Other MAbs two-site combinations (Adn94-Adn63, Adn279-Adn94, Adn214-Adn27, Adn222-Adn94, Adn305-Adn279) react identically with antigen in serum and plasma identically.

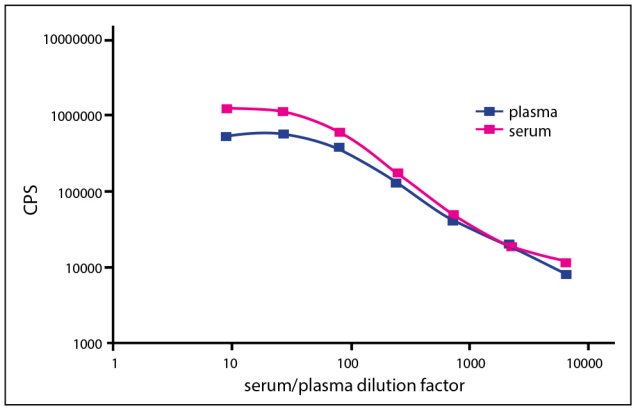


FIGURE 3. Normal human serum titration curve in sandwich immunofluorescent assay. *Adn94 MAb was used as a coating antibody (1 µg/well), MAb Adn63 was used as a detection antibody (0.2 µg/well). Normal human serum, serially diluted with phosphate- buffered saline (10 mM K-phosphate, pH 7.4, 150 mM NaCl, 0.1% Tween-20) was used as an antigen.*

Recognition of adiponectin by assays Adn20- Adn23 and Adn36-Adn27 in serum is Ca2+-sensitive (Fig. 5). Chelating of Ca2+ ions by EGTA leads to the rearrangements in adiponectin structure and changes in the interaction of one of the antibodies with the antigen. Other assays do not demonstrate Ca2+-dependence in the antigen recognition and react identically with adiponectin in serum or citrate plasma.

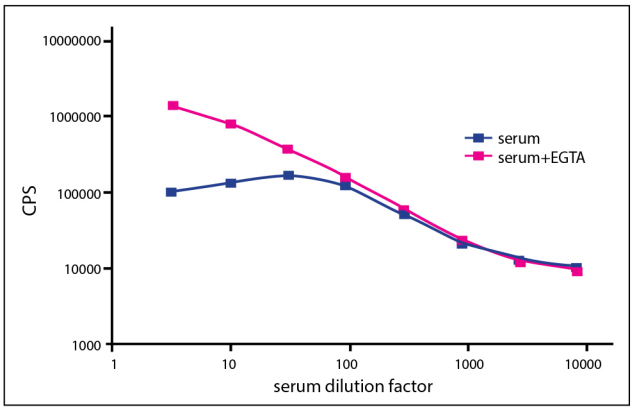


FIGURE 5. Serum titration curve for the assay Adn20-Adn23. *Pooled normal human serum was serially diluted with phosphate-buffered saline with EGTA or w/o EGTA (10 mM K-phosphate, pH 7.4, 150 mM NaCl, 0.1% Tween-20, 10 mM EGTA).*

Western blotting

All MAbs were tested on their ability to recognize adiponectin in Western blotting. Only five the of tested antibodies – MAbs Adn20, Adn23, Adn63, Adn214 and Adn222 – reacted with adiponectin transferred onto nitrocellulose membrane after SDS-PAGE in reducing conditions (Fig. 6).

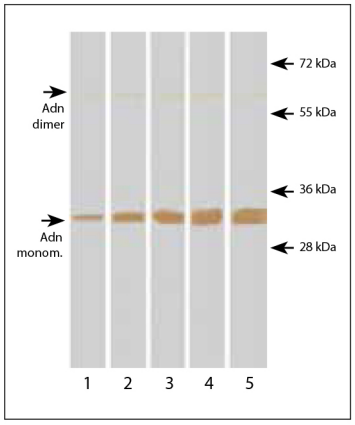


Figure 6. Immunodetection of native adiponectin with anti-Adn MAbs in Western blotting after SDS-electrophoresis in reducing conditions.
40 ng of native purified adiponectin was loaded onto each track, nitrocellulose membrane was stained with 5 µg/ml of various anti-adiponectin MAbs in phosphate-buffered saline, containing 5% dry milk and 0.1% Tween-20.
1: Adn20, 2: Adn23, 3: Adn63, 4: Adn214, 5: Adn222, MW markers are marked by arrows.

Assays detecting total, HMW or LMW formof human adiponectin

To establish an assay oligomer specificity, serum proteins were separated according to their molecular masses by means of size-exclusion chromatography and immunoreactivity in fractions was measured. The assay Adn20-Adn23 detects two oligomeric forms of adiponectin: mostly HMW and to a lesser extent, the MMW form (Fig. 7A). The assay Adn94-Adn63 recognizes all three Adn oligomeric forms - total adiponectin (Fig. 7B) and the assay Adn214-Adn27 reacts primarily with the LMW form of adiponectin (Fig. 7C).

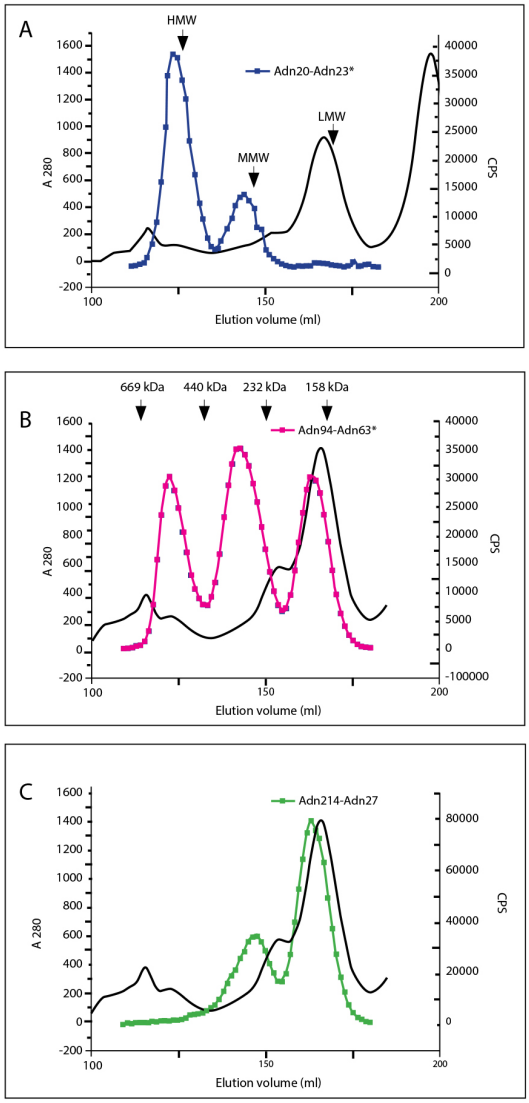


Figure 7. Sandwich ELISA in protein fractions after size-exclusion chromatography, measured by three different capture-detection antibody combinations. *(A) Adn20-Adn23, (B) Adn94-Adn63 and (C) Adn214-Adn27. 1 ml of normal human serum was applied onto the column. Positions of oligomeric forms of adiponectin and molecular weight markers are depicted in the picture. The black line presents the optical density detected at 280 nm*

Native purified adiponectin

Native adiponectin purified from normal human plasma is the best calibrator for immunoassays. Native adiponectin was isolated from normal human plasma using a combination of chromatographic methods. Its purity is approximately 95%.

Native purified adiponectin fully recovers its immunoreactivity after lyophilization and reconstitution by the addition of deionized water (Fig. 8).

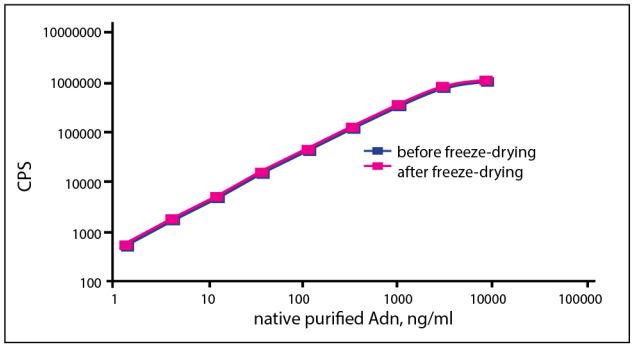


FIGURE 8. Lyophilization does not affect immunological activity of native purified adiponectin measured by assay Adn94-Adn63.

Purified native adiponectin contains all three oligomeric forms of Adn (Fig. 9) and can therefore serve as a calibrator for all types of Adn assays: total Adn, HMW- or LMW-specific.

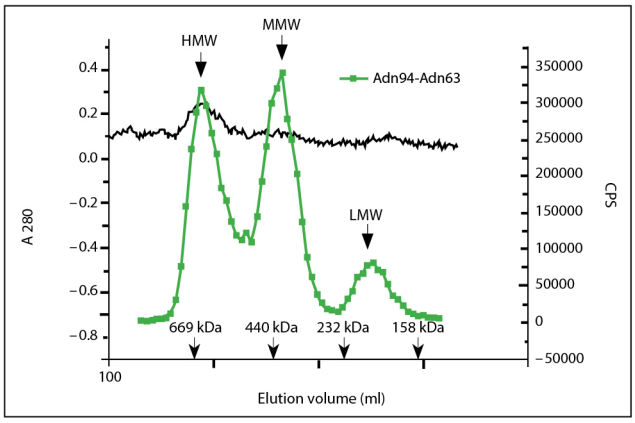


FIGURE 9. Native purified adiponectin contains all oligomeric forms. *3 µg of adiponectin was a1p50plied onto a gel-filtra-2t0i0on column and immunoreactivity in fractions was measured by the sandwich ELISA using Adn94 and Adn63 as capture and detection antibodies respectively. Molecular weight markers are depicted by arrows on the x-axis. The black curve represents the optical density measured at 280 nm.*

Ordering Information:

MONOCLONAL ANTIBODIES

Product	Cat #	MAb	Subclass	Remarks
Adiponectin, human	1-ADP	Adn20	IgG2a	EIA, WB
		Adn23	IgG2a	EIA, WB
		Adn27	IgG2a	EIA
		Adn36	IgG2a	EIA
		Adn63	IgG1	EIA, WB
		Adn94	IgG1	EIA
		Adn214	IgG1	EIA, WB
		Adn222	IgG1	EIA, WB
		Adn279	IgG1	EIA
		Adn305	IgG1	EIA

ANTIGEN

Product	Cat #	Purity	Source
Adiponectin, human	8-ADP	>95%	Pooled human plasma

References

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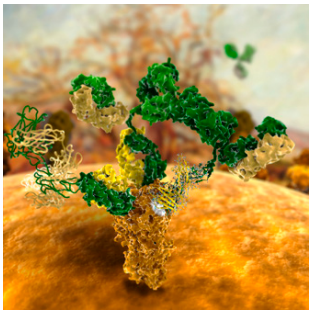
ASSAY NOTES
Product Information

Courtesy of HyTest, Ltd

HbA1c - The Glycated Isoform of Hemoglobin



Diabetes is one of the most challenging health problems in the 21st century and it is among leading causes of death in most developed economies but alarmingly also in developing economies. Diabetes causes substantial health costs because of complications often associated with it such as coronary artery and peripheral vascular disease, stroke, diabetic neuropathy, amputations, renal failure and blindness (1).

Type 2 diabetes is defined by the presence of markedly elevated levels of plasma glucose (hyperglycemia). Repeated glucose measurements are an essential part of diabetes daily care but other tools are needed to assess patients' long term glycemic control as blood glucose levels fluctuate markedly during the day. Number of studies have shown that glycated hemoglobin HbA1c concentration (percentage of HbA1c to total hemoglobin content) provides a better estimate of average hyperglycemia than routine determinations of blood glucose concentration, and it is the most widely used index of chronic hyperglycemia (2). HbA1c content provides information about glucose concentration in the blood during last 2-3 months and therefore it has been used to monitor treatment in patients with type 2 diabetes.

CLINICAL UTILITY

- **Chronic hyperglycemia**
- **Type 2 diabetes**

Glycation of proteins in blood circulation is a non-enzymatic process of glucose addition to proteins and majority of proteins are glycated in the bloodstream and therefore long-lived blood proteins may serve as an indirect estimate of glucose concentration in the bloodstream. Hemoglobin is an intracellular tetrameric protein whose lifespan is around 120 days. Hemoglobin consists of 4 polypeptide chains: 2 α-globins and 2 β-globins. It is known that hemoglobin has several amino acid residues which may be glycated, but only glycation of α-NH2-group of N-terminal valine leads to a change of overall charge of the hemoglobin making it possible to use ion-exchange chromatography for HbA1c measurements (5). Chromatographic methods give accurate results but these cannot be used in point-of-care setting and therefore there is a need for reliable immunodiagnostic methods.

Advanced ImmunoChemical announces a monoclonal antibody specic to HbA1c that may be used both in direct and sandwichELISA.

Anti-human HbA1c monoclonal antibodies

Hybridoma clones were derived from the hybridization of Sp2/0 myeloma cells with spleen cells of Balb/c mice immunized with glycated synthetic peptide. Speci city of antibody has been checked in direct ELISA. Hb6 antibody reacts with both HbA1c and HbA10, whereas 75C9 detects only HbA1c (Fig. 1).

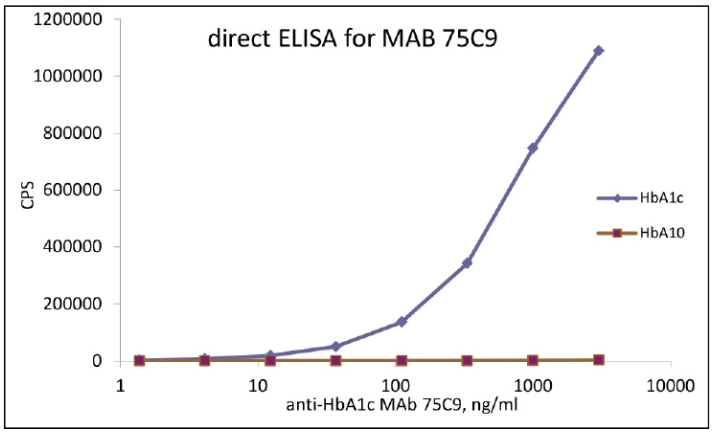


FIGURE 1. Specificity of MAb 75C9 interaction with HbA1c checked by direct ELISA. *Native HbA1c or HbA10 were used as a coating (100 ng/well), MAb 75C9 was labeled with stable Eu^{3+} chelate and served as detection.*

MAbs selected were tested in sandwich fluoroimmunoassay and MAb pair Hb6-75C9 was shown to detect specifially native glycated hemoglobin HbA1c (Fig. 2).

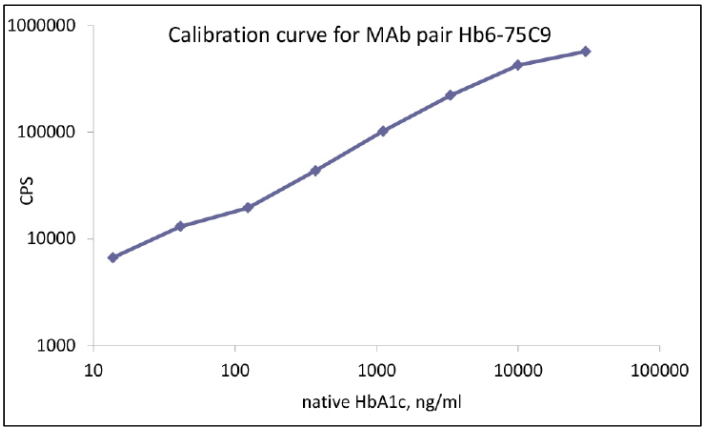


FIGURE 2. Calibration curve for Hb6-75C9 fluoroimmunoassay. *MAb Hb6 was used as a coating (1 µg/well), MAb 75C9 was labeled with stable Eu^{3+} chelate and served as detection (0.4 µg/well). Native HbA1c was utilized as an antigen.*

Ordering Information:

MONOCLONAL ANTIBODIES

Product	Cat #	MAb	Subclass	Remarks
Hemoglobin, human, $HbA1_0$	2-HH10	Hb4	IgG1	EIA
		Hb6	IgG1	EIA
Hemoglobin, human, glycated, $HbA1_c$	2-GhH	75C9	IgG1	EIA

References

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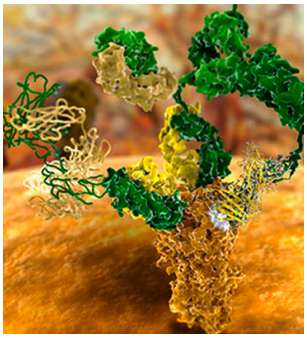
Courtesy of HyTest, Ltd

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 process- ing) (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 consid- erable 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).

CLINICAL UTILITY

- **Diabetes mellitus**
- **Hypoglycemia**

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 proinsulin. **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.

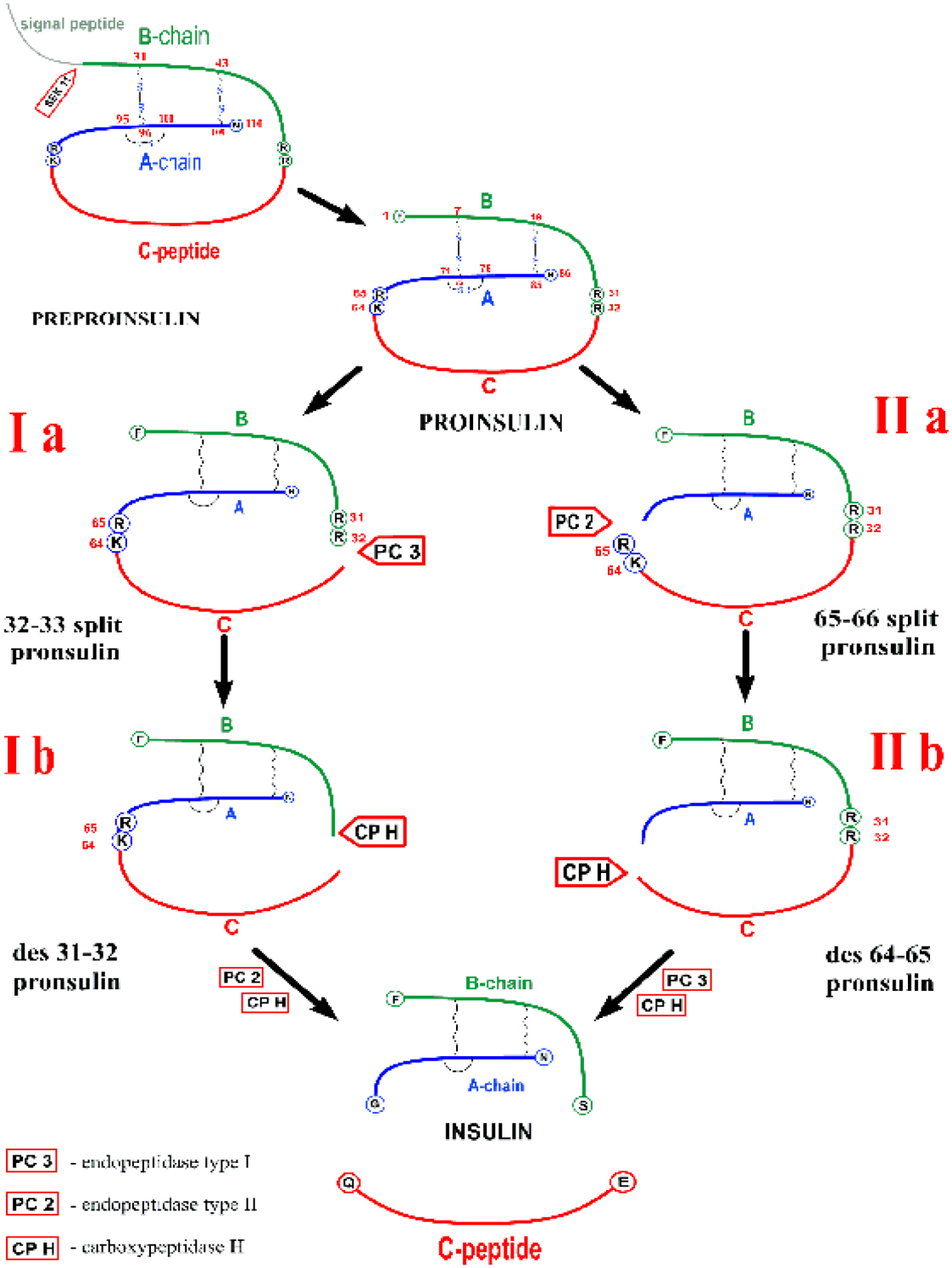


Figure 1. Processing of proinsulin

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

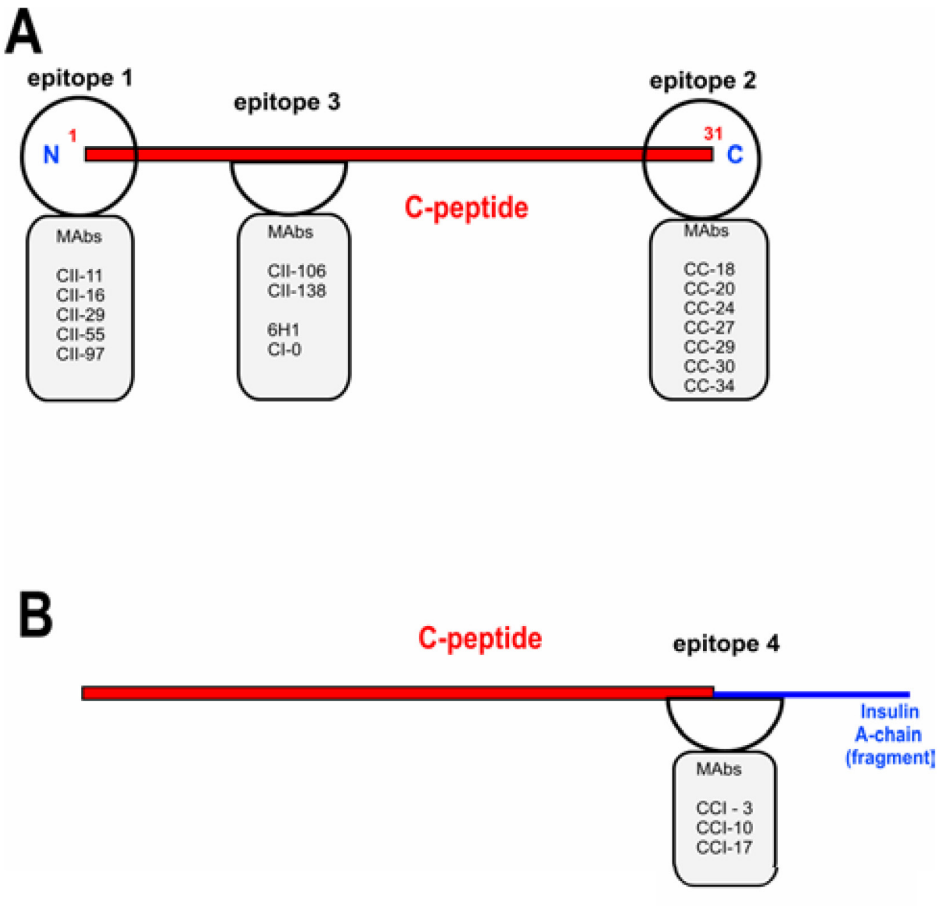


Figure 2. Epitope specificity of anti-rat C-peptide antibodies.

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 anti- bodies 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).

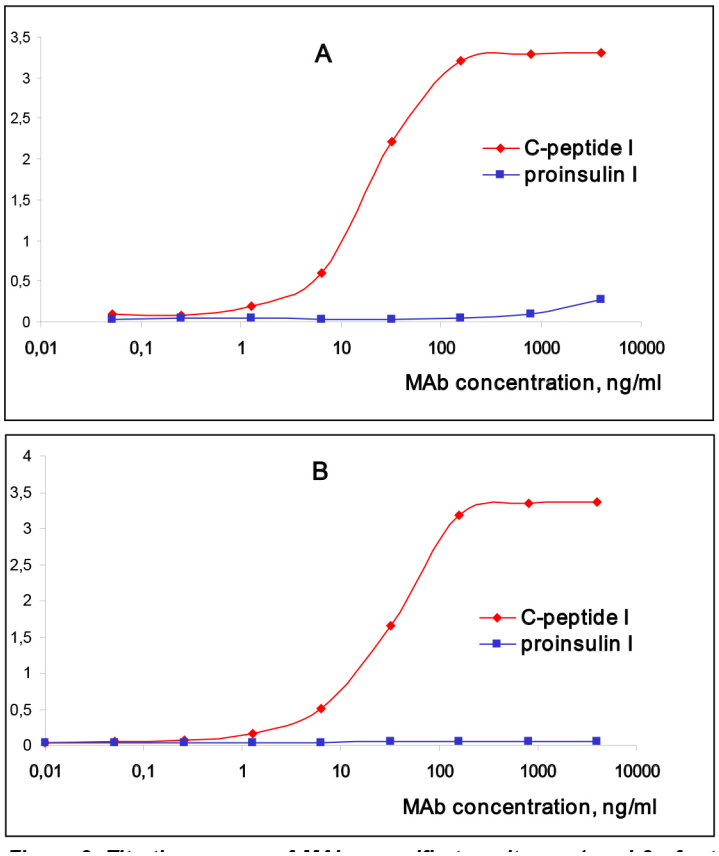
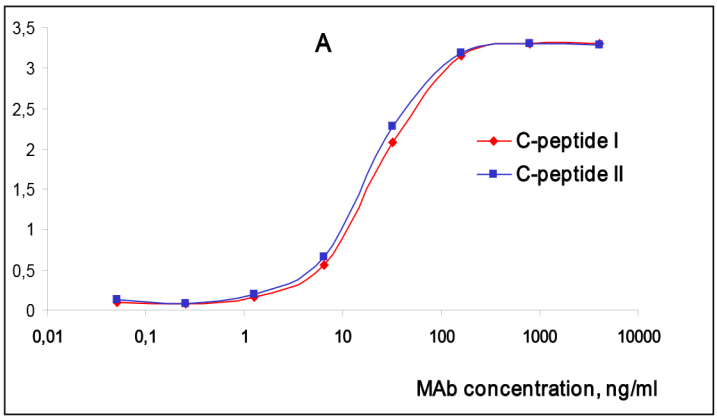
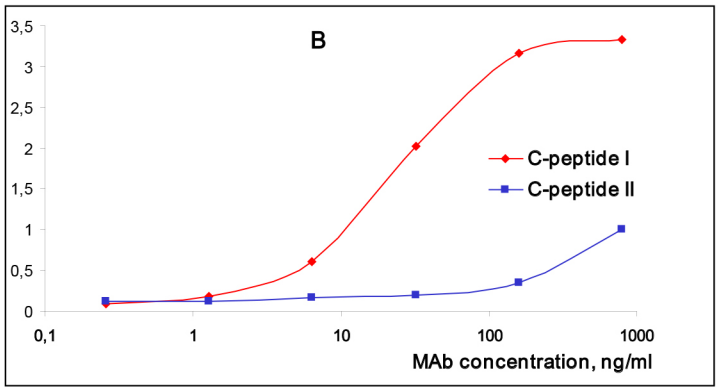


Figure 3. Titration curves of MAbs specific to epitopes 1 and 2 of rat C-peptide.
A. MAb CII-16 (epitope 1)
Antigens:
• N-terminal part of rat C-peptide I conjugated with the carrier protein: 0.04 µg/well
• rat proinsulin I: 0.04 µg/well
B. MAb CC34 (epitope 2)
Antigens:
• rat C-peptide I: 0.01 µg/well
• 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.





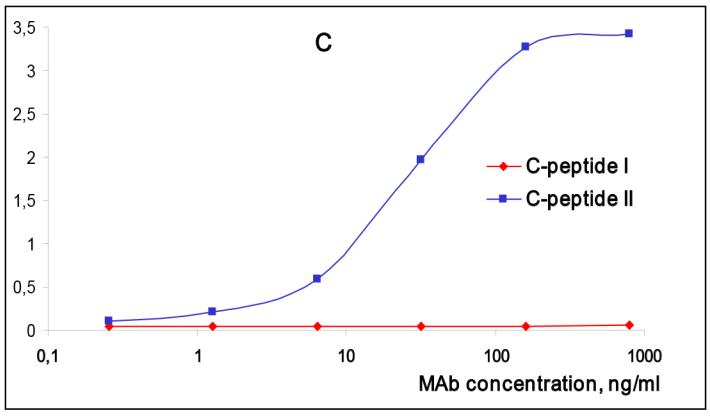


Figure 4. Titration curves of monoclonal antibodies specific to different isoforms of rat C-peptide.
A. Titration curve for MAb CII-29 (detects both C-peptides I and II)
B. Titration curve for MAb 6H1 (C-peptide I specific)
C. Titration curve for MAb CII-106 (C-peptide II specific)
Antigens:
• N-terminal 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%.

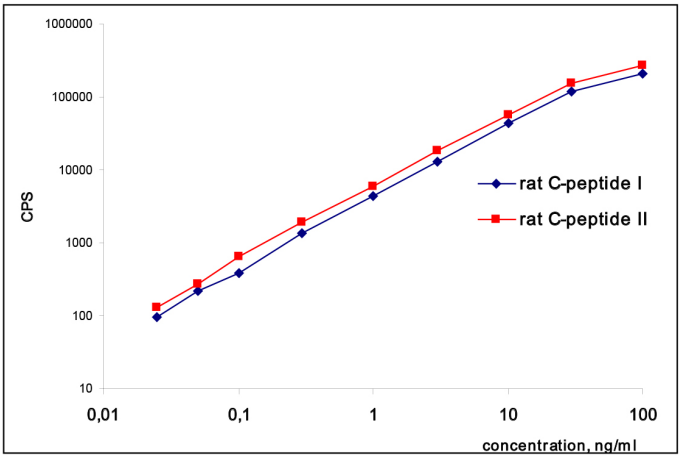


Figure 5. Calibration curve for one step rat C-peptide immunoassay.
Capture MAb (biotinylated): CC24, 0.4 µg/well Detection MAb (Eu-labeled): CII-29, 0.4 µg/well Streptavidin coated plates
Incubation time: 30 min

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 measure- ments 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

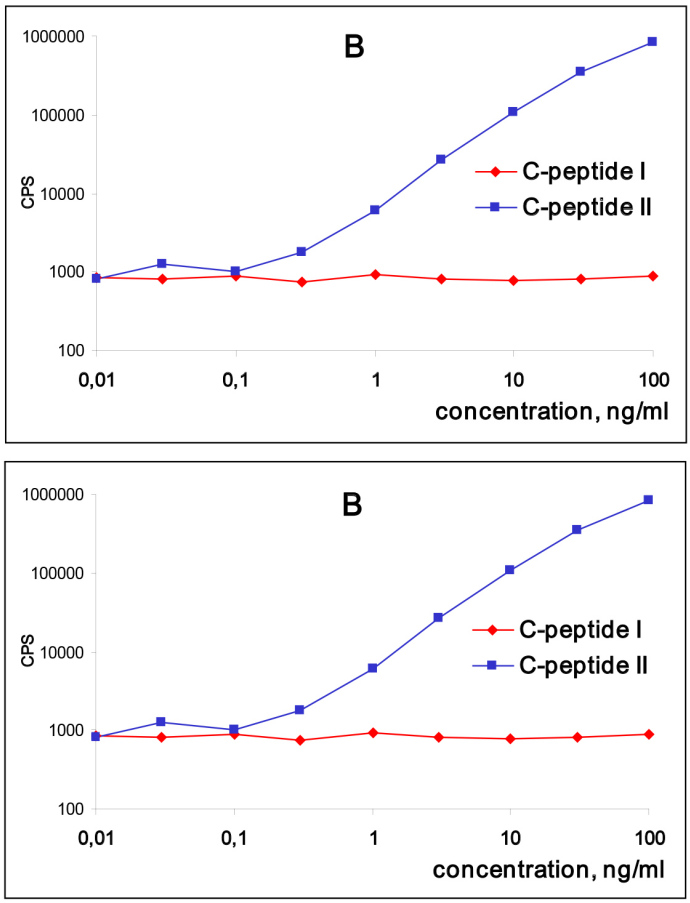


Figure 6. Calibration curve for separate rat C-peptide I and C-peptide II immunodetection in sandwich immunoassay.
A. Rat C-peptide I immunodetection.
Capture MAb: CC18, 1 µg/well
Detection MAb (Eu-labeled): 6H1, 0.2 µg/well Incubation time: 30 min
B. Rat C-peptide II immunodetection.
Capture MAb: CC34, 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)

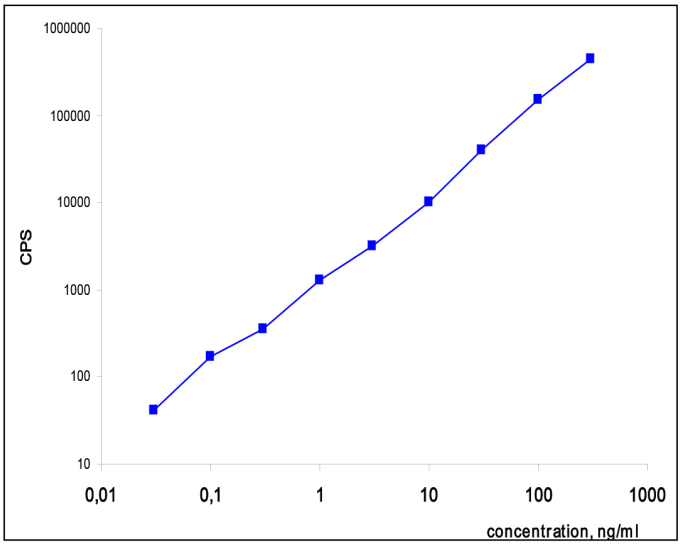


Figure 7. Calibration curve for rat proinsulin I immunodetection in one step sandwich immunoassay.
Capture MAb (biotinylated): CCI-17, 0.2 µg/well
Detection MAb (Eu-labeled, Cat.# 2I3): CI-0: 0.2 µg/well
Incubation time: 30 min

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).

References

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Courtesy of HyTest, Ltd

Retinol-binding protein 4 (RBP4)

TYPE 2 Diabetes

Retinol-binding protein 4 (RBP4) belongs to a lipocalin protein family and functions as a carrier protein for vita- min A in serum. Human retinol-binding protein circulating in blood consists of 183 amino acid residues. Several truncated isoforms of RBP4 lacking 1, 2, 4 or 6 of the very C-terminal residues were alsodescribed in literature (7). In blood RBP4 carries retinol (vitamin A) which is bound to RBP4 in equimolar ratio. Besides, a major part of circulating RBP4 forms complex with prealbumin (transthyretin) and according to Jaconi et al. only a small fraction of free RBP4 can be found in serum. (7)

RBP4 has been studied since the 1960s, mainly as a transporter of retinol. However, recent data suggests that RBP4 may contribute to pathogenesis of type 2 diabetes. Yang et al. demonstrated that serum RBP4 levels are elevated in patients with obesity and type 2 diabetes. Studies in mice showed that serum RBP4 may cause insulin resistance (1). Therefore, while on the one hand there is a growing body of evidence demonstrating that RBP4 is a promising marker of the risk of type 2 diabetes, on the other hand there is a conflicting situation in the literature regarding RBP4 clinical utility in terms of predicting insulin resistance and type 2 diabetes (3). Some authors show a strict correlation between circulating RBP4 and magnitude of insulin resistance in subjects with obesity and type 2 diabetes and non-obese subjects with a family history of type 2 diabetes (2). On the contrary, others (4, 5) had not found any correlation between those variables. This confusing situation could at least partially be explained by the heterogeneity of the RBP4 in serum and by methodological shortcomings in determining level of circulating RBP4 (6). If epitope of diagnostic antibody is influenced by RBP4 truncation or by complex formation with retinol or prealbumin, then the level of RBP4 determined by the assay, utilizing such an antibody, would be different from the results of measurements by the assays with antibodies that are not susceptible to such modifications.

Advanced ImmunoChemical offers a set of mouse monoclonal anti-human RBP4 antibodies that are suitable for the development of sandwich immunoassays for the quantitative detection of circulating RBP4 in human plasma as well as for the immunodetection of RBP4 in direct ELISA, Western blotting or that can be used for the immunoprecipitation of the antigen.

1. Purified endogenous RBP4

Native RBP4 represents the most natural form of RBP4 and is therefore the antigen of choice for assay calibration. It is known that in serum RBP4 exist mostly as a 1:1 complex with prealbumin (transthyretin) and only a small part of RBP4 in the blood is presented as a free form. (7)

Advanced ImmunoChemical offers two types of purified native RBP4 antigen: free and complexed with prealbumin. Both forms of endogenous RBP4 (free and complexed) were purified from normal human serum in mild conditions using several chromatographic steps (Fig. 1).

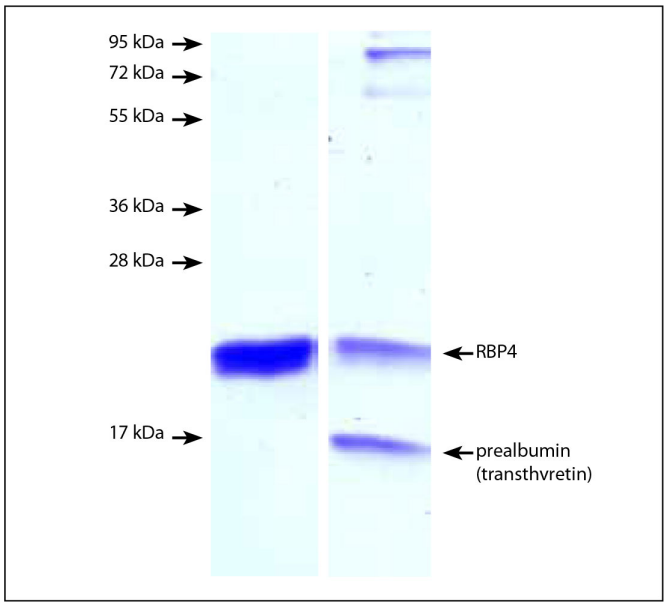

Figure 1. RBP4 isolated from normal human serum (coomassie-stained gel after SDS-electrophoresis in reduced conditions).
Lanes:
1: free native RBP4, 3 µg per track
2: native RBP4 complexed with prealbumin, 2 µg of total protein per track Molecular weight marker positions are marked by arrows.

Both native free and native complexed RBP4 antigens are unaffected by multiple (at least 5 - 7) freeze-thaw cycles (Fig. 2).

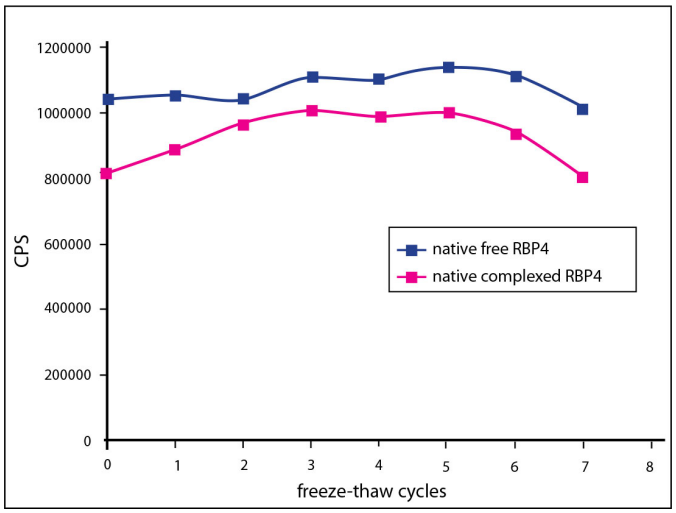

Figure 2. Immunoreactivity of native free and native complexed RBP4 after several freeze-thaw cycles measured with the assay RB48 - RB42. *Capture antibody: RB48 (1 µg/well) Detection antibody: RB42 labeled with stable Eu3+ chelate (0.2 µg/well) Antigen: Native isolated RBP4.*

Ordering Information:

Product	Cat #	Purity	Source
Retinol-binding protein 4 (RBP4) from human plasma, complexed with prealbumin	8-RBP4-c	>70%	human plasma
Retinol-binding protein 4 (RBP4) from human plasma, free	8-RBP4-f	>95%	human plasma

2. Anti-human RBP4 monoclonal antibodies

Host animal:	Mice Balb/c
Cell line used for fusion:	Sp2/0
Antigen:	Human recombinant retinol-binding protein
Purification method:	Protein A affinity chromatography
Presentation:	MAbs solution in PBS with 0.1% sodium azide
Application:	ELISA, RBP4 sandwich immunoassay, Western blotting

Hybridoma clones have been derived from the hybridization of Sp2/0 myeloma cells with spleen the cells of Balb/c mice immunized with human recombinant RBP4.

2.1 Applications

2.1.1. Western blotting

MAbs RB42, RB45, RB48, RB51 can be used for RBP immunodetection in Western blotting (Fig. 3).

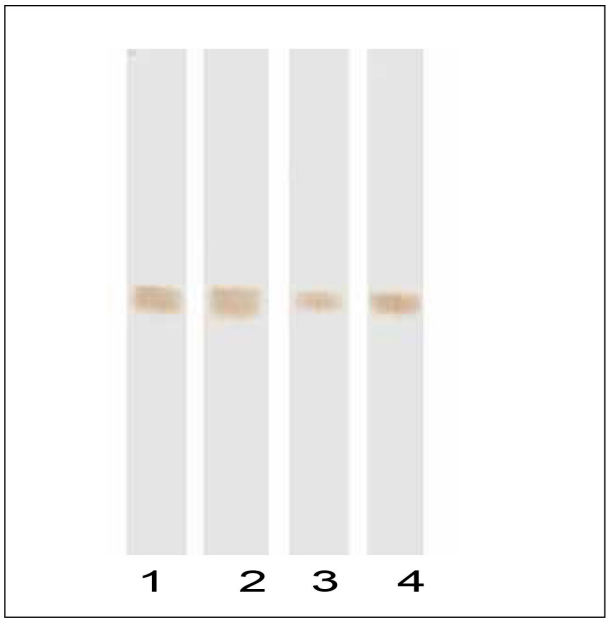

Figure 3. Immunodetection of RBP4 in Western blotting after SDS- electrophoresis in reducing conditions by MAb RB42 (lane 1), RB45 (lane 2), RB48 (lane 3) and RB51 (lane 4).
1 µg per track of purified endogenous RBP4 was loaded onto gel.

2.1.2. Immunoprecipitation

Anti-RBP4 MAbs being immobilized onto BrCN-activated Sepharose can be used as an affinity matrix for the immunoprecipitation of RBP4.

2.1.3. Sandwich immunoassay for RBP4 detection in human plasma

Anti-human RBP4 MAbs were obtained after mice immunization with human recombinant RBP4. All MAbs were tested in direct ELISA with human recombinant and native (endogenous, purified from human blood) RBP4. The best MAbs were further tested in sandwich immunoassay and several two-site combinations demonstrating the highest sensitivity for both recombinant and endogenous proteins were selected (Fig. 4) and recommended by our specialists for the development of RBP4 sandwich immunoassays.

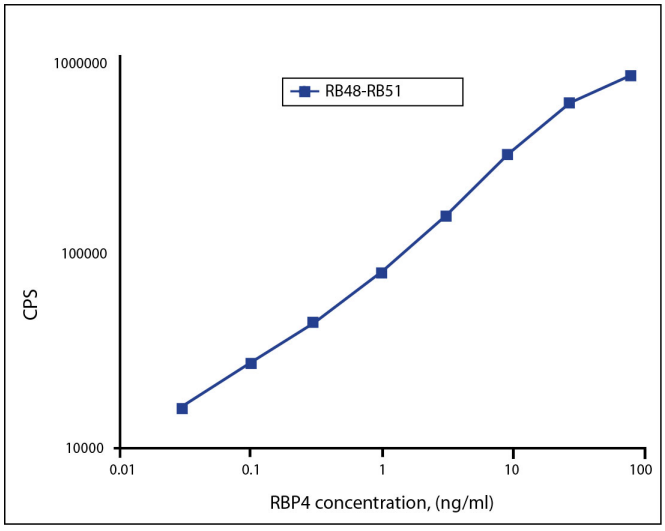

Figure 4. Calibration curve of RBP4 sandwich immunoassay.
Capture antibody: RB48 (1 µg/well)
Detection antibody: RB51 labeled with stable Eu3+ chelate (0.2 µg/well) Antigen: Purified endogenous RBP4.

Advanced ImmunoChemical offers MAbs RB42, RB48, RB45, RB49, RB51 and RB55, that are suitable for the immunodetection of native RBP4 in direct ELISA and sandwich immunoassay.

Recommended combinations of antibodies for the development of sandwich immunoassay are (capture -detection):

RB48 - RB42
RB48 - RB49
RB48 - RB51
RB55 - RB45

Selected assays recognize endogenous antigen in highly diluted human plasma (Fig. 5)

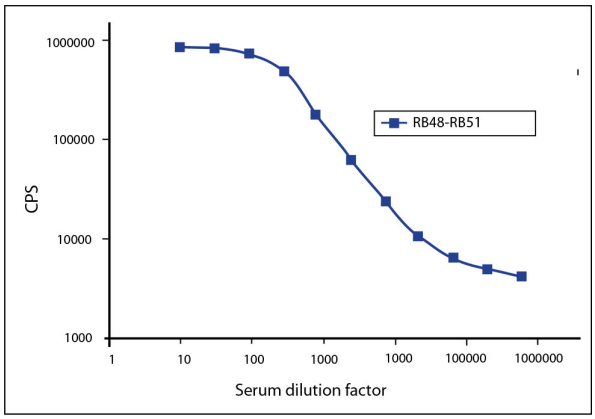

Figure 5. Titration curve of human plasma sample.
Capture antibody: RB48 (1 µg/well)
Detection antibody: RB51 labeled with stable Eu^{3+}chelate (0.2 µg/well)
Antigen: Normal human serum diluted with PBS containing 0.1% Tween-20.

Immunoreactivity of native RBP4, being measured by recommended MAb combinations, is unchanged in the presence of EDTA in the tested sample (Fig. 6).

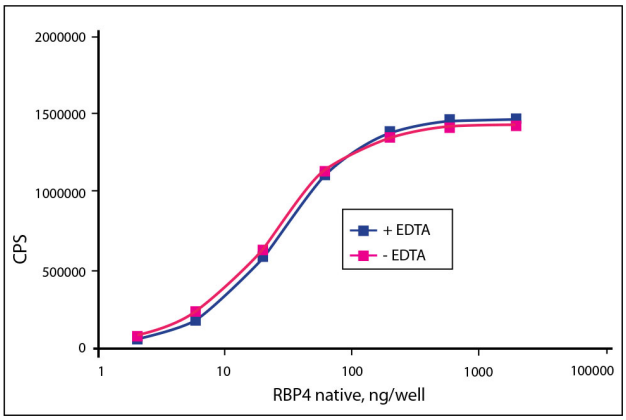

Figure 6. Immunodetection of purified endogenous RBP4 in sandwich immunoassay by RB48-RB42 MAb assay in presence of 5 mM EDTA (blue line) or in absence of EDTA (pink line).
Capture antibody: RB48 (1 µg/well)
Detection antibody: RB42 labeled with stable Eu^{3+}chelate (0.2 µg/well)
Antigen: Native isolated RBP4

All anti-RBP4 MAbs recognize both free RBP4 and RBP4 complexed with prealbumin.

Ordering Information:

MAb	Cat #	Specificity	Subclass	Application
RB42	2-RBP4	Retinol-binding protein 4 (RBP4)	IgG1	EIA, WB
RB45		Retinol-binding protein 4 (RBP4)	IgG1	EIA, WB
RB48		Retinol-binding protein 4 (RBP4)	IgG1	EIA, WB
RB49		Retinol-binding protein 4 (RBP4)	IgG1	EIA, WB
RB51		Retinol-binding protein 4 (RBP4)	IgG1	EIA, WB
RB55		Retinol-binding protein 4 (RBP4)	IgG1	EIA, WB

References

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Leptin

Leptin is a hormone that is secreted by adipocytes. It crosses the blood-brain barrier and binds to receptors in the brain. When the level of leptin increases, it tells the brain that the energy reservoirs are full and that there is no need for food intake. This signaling route is often impaired in obese people as a result of leptin resistance.

We provide monoclonal antibodies that are specific to leptin for research purposes. They have been tested in sandwich immunoassays and Western blotting.

Monoclonal Antibodies

Cat #	Product	Tested Application
1-L1	Monoclonal mouse anti-human leptin	Enzyme immunoassays Western blotting

Ghrelin

Ghrelin is a hormone that is secreted by cells in the gastrointestinal track. Both leptin and ghrelin regulate the appetite but in an opposite manner to leptin, ghrelin increases the sensation of hunger. It crosses the blood-brain barrier and binds to receptors on hypothalamus.

We provide monoclonal antibodies that are specific to ghrelin for research purposes. They have been tested in ELISA

Monoclonal Antibodies

Cat #	Product	Tested Application
1-G1	Monoclonal mouse anti-ghrelin	Enzyme immunoassays

References

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