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(54) Method of selectively assaying adiponectin multimers

(57) A method for selectively assaying a target adiponectin multimer in a biological sample, comprising a step of distinguishing target adiponectin multimer from the other adiponectin multimers by using a protease and an antibody to assay immunologically, wherein the adiponectin multimer is derived from human blood, and is of the following four types of adiponectin, and one or two of the four types of adiponectin are selected from the total adiponectin and immunoassayed, through use of a protease and an antibody;

(1) ULMW-Ad: exhibits the highest mobility among the four main stained bands detected when adiponectin which has been purified from human serum or human plasma is electrophoresed on polyacrylamide gel (2 to 15%) under non-denaturing conditions, and has a molecular weight around 100 kDa as measured through SDS-PAGE after intramolecular crosslinking;

(2) LMW-Ad: exhibits the second highest mobility, next to ULMW-Ad, among the four main stained bands detected when adiponectin which has been purified from human serum or human plasma is electrophoresed on polyacrylamide gel (2 to 15%) under non-denaturing conditions, has a molecular weight around 150 kDa as measured through SDS-PAGE after intramolecular crosslinking, and binds to albumin via a disulfide bond;

(3) MMW-Ad: exhibits the third highest mobility, next to LMW-Ad, among the four main stained bands detected when adiponectin which has been purified from human serum or human plasma is electrophoresed on polyacrylamide gel (2 to 15%) under non-denaturing conditions, and has a molecular weight around 250 kDa as measured through SDS-PAGE after intramolecular crosslinking; and

(4) HMW-Ad: exhibits the lowest mobility among the four main stained bands detected when adiponectin which has been purified from human serum or human plasma is electrophoresed on polyacrylamide gel (2 to 15%) under non-denaturing conditions, and has a molecular weight of 300 kDa or higher as measured through SDS-PAGE after intramolecular crosslinking.

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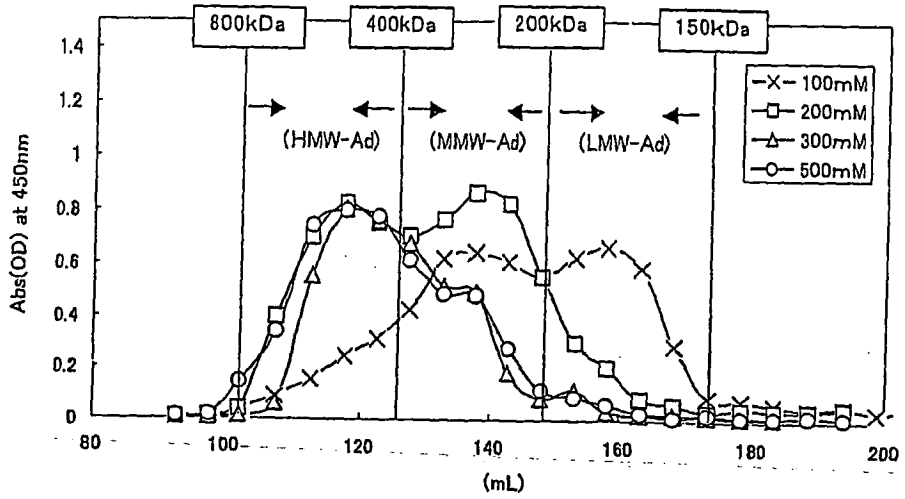
(Cont. next page)

[Fig. 1]


800kDa
400kDa
200kDa
150kDa
(HMW-Ad)
(MMW-Ad)
(LMW-Ad)
100mM
200mM
300mM
500mM
Abs(OD) at 450nm
1.4
1.2
1
0.8
0.6
0.4
0.2
0
80
100
120
140
160
180
200
(mL)

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EUROPEAN SEARCH REPORT

Application Number
EP 09 00 4308

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
D,X	PAJVANI U B ET AL: "Structure-function studies of the adipocyte-secreted hormone Acrp30/adiponectin. Implications for metabolic regulation and bioactivity" JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOLOCHEMICAL BIOLOGISTS, BIRMINGHAM,, US, vol. 278, no. 11, 14 March 2003 (2003-03-14), pages 9073-9085, XP002251775 ISSN: 0021-9258 * figures 2A,3C,5C,E,6C,D * page 9080, last paragraph to page 9081, left column. * figure 4 *	1-9	INV. G01N33/53 G01N27/447
Y	WAKI H ET AL: "Impaired Multimerization of Human Adiponectin Mutants Associated with Diabetes" JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOLOCHEMICAL BIOLOGISTS, BIRMINGHAM, US, vol. 278, no. 41, 10 October 2003 (2003-10-10), pages 40352-40363, XP002988190 ISSN: 0021-9258 * abstract * * pages 40352-53 * * pages 40361-62 *	10-15	TECHNICAL FIELDS SEARCHED (IPC) G01N

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The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
The Hague	29 May 2009	Lunter, Pim

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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A : technological background
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T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
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& : member of the same patent family, corresponding document

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EUROPEAN SEARCH REPORT

Application Number
EP 09 00 4308

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
Y	TSAO TAU-SHUEN ET AL: "Role of disulfide bonds in Acrp30/adiponectin structure and signaling specificity. Different oligomers activate different signal transduction pathways." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 278, no. 50, 30 September 2003 (2003-09-30), pages 50810-50817, XP002408187 ISSN: 0021-9258 page 50817, last paragraph. Note on-line pub. date * figure 5 * -----	10-15	
			TECHNICAL FIELDS SEARCHED (IPC)

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专利名称(译)	选择性测定脂联素多聚体的方法		
公开(公告)号	EP2071333A3	公开(公告)日	2009-07-08
申请号	EP2009004308	申请日	2004-10-15
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发明人	EBINUMA, HIROYUKI YAGO, HIROKAZU AKIMOTO, YUKA MYAZAKI, OSAMU KADOWAKI, TAKASHI YAMAUCHI, TOSHIMASA HARA, KAZUO		
IPC分类号	G01N33/53 G01N27/447 G01N33/74		
CPC分类号	G01N33/74 G01N2800/042 G01N2800/32		
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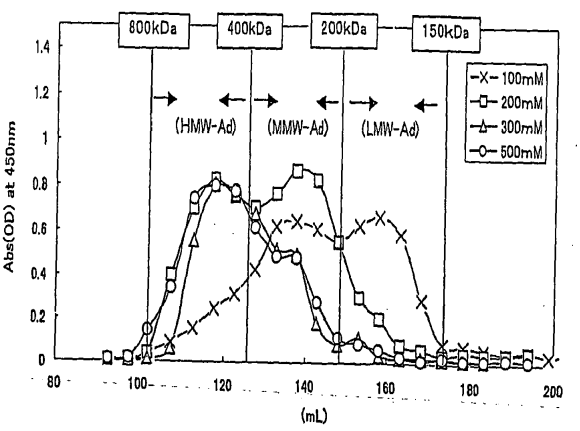
其他公开文献	EP2071333B1 EP2071333A2		
外部链接	Espacenet		

摘要(译)

一种选择性测定生物样品中的目标脂联素多聚体的方法，包括通过使用蛋白酶和免疫学测定抗体来区分目标脂联素多聚体与其他脂联素多聚体的步骤，其中脂联素多聚体来源于人血液，并且是通过使用蛋白酶和抗体，从脂联素总和免疫测定中选择以下四种脂联素中的一种或两种，并且四种脂联素中的一种或两种；（1）ULMW-Ad：在人体血清或人血浆中纯化的脂联素在非变性条件下在聚丙烯酰胺凝胶（2-15%）上进行电泳时检测到的四个主要染色条带中具有最高的迁移率，并且具有在分子内交联后通过SDS-PAGE测量的分子量约为100kDa；（2）LMW-Ad：在脂联素检测到的四个主要染色带中，在ULMW-Ad旁边显示出第二高的迁移率从人血清中纯化或人血浆在非变性条件下在聚丙烯酰胺凝胶（2-15%）上进行电泳，分子量交联后通过SDS-PAGE测定的分子量约为150kDa，并通过二硫键与白蛋白结合；（3）MMW-Ad：在从人血清或人血浆中纯化的脂联素在聚丙烯酰胺凝胶（2-15%）下电泳后检测到的四

[Fig. 1]



个主要染色条带中，显示出LMW-Ad旁边第三高的迁移率。非变性条
件，在分子内交联后通过SDS-PAGE测得的分子量约为250kDa;（4）HMW-Ad：在人体血清或人血浆中纯化的脂联素在非变性条件下在聚丙烯酰胺凝胶（2-15%）上进行电泳时检测到的四个主要染色条带中的移动性最低，并具有在分子内通过SDS-PAGE测量的分子量为300kDa或更高交联。