



(12) Translation of
European patent specification

(11) NO/EP 2952577 B1

NORWAY

(19) NO
(51) Int Cl.
C12N 5/071 (2010.01)
C12N 1/00 (2006.01)
C12N 5/07 (2010.01)

Norwegian Industrial Property Office

- (21) Translation Published 2019.09.23
- (80) Date of The European Patent Office Publication of the Granted Patent 2019.06.19
- (86) European Application Nr. 14745765.9
- (86) European Filing Date 2014.01.31
- (87) The European Application's Publication Date 2015.12.09
- (30) Priority 2013.02.01, JP, 2013018774
- (84) Designated Contracting States: AL ; AT ; BE ; BG ; CH ; CY ; CZ ; DE ; DK ; EE ; ES ; FI ; FR ; GB ; GR ; HR ; HU ; IE ; IS ; IT ; LI ; LT ; LU ; LV ; MC ; MK ; MT ; NL ; NO ; PL ; PT ; RO ; RS ; SE ; SI ; SK ; SM ; TR
- (73) Proprietor Tohoku University, 2-1-1, Katahira Aoba-ku, Sendai-shi, Miyagi 980-8577, Japan
Meiji Seika Pharma Co., Ltd., 4-16, Kyobashi 2-chome Chuo-ku, Tokyo 104-8002, Japan
National University Corporation Tokyo University of Agriculture and Technology, 3-8-1, Harumi-cho, Fuchu-shi, Tokyo 183-8538, Japan
- (72) Inventor GOTO, Masafumi, c/o TOHOKU UNIVERSITY2-1-1 KatahiraAoba-ku, Sendai-shiMiyagi 980-8577, Japan
MURAYAMA, Kazutaka, c/o TOHOKU UNIVERSITY2-1-1 KatahiraAoba-ku, Sendai-shiMiyagi 980-8577, Japan
YAMAGATA, Youhei, c/o National University Corporation TokyoUniversity of Agriculture and Technology3-8-1 Harumi-cho, Fuchu-shiTokyo 183-8538, Japan
WATANABE, Kimiko, c/o TOHOKU UNIVERSITY2-1-1 KatahiraAoba-ku, Sendai-shiMiyagi 980-8577, Japan
- (74) Agent or Attorney BRYN AARFLOT AS, Stortingsgata 8, 0161 OSLO, Norge
-

(54) Title **METHOD FOR SEPARATING CELL FROM BIOLOGICAL TISSUE**

(56) References Cited:
WO-A1-2012/124338
JP-A- H09 508 026
CA-A1- 2 830 140
WO-A1-2010/058707
US-A- 5 830 741
US-A- 5 753 485

MONICA SALAMONE ET AL: "Biochemical Comparison Between Clostridium Hystoliticum Collagenases G and H obtained by DNA Recombinant and Extractive Procedures", CHEMICAL ENGINEERING TRANSACTIONS, vol. 27, 1 January 2012 (2012-01-01), pages 259-264, XP055280422,

BRANDHORST D ET AL: "Adjustment of the Ratio Between Collagenase Class II and I Improves Islet Isolation Outcome", TRANSPLANTATION PROCEEDINGS, ELSEVIER INC, ORLANDO, FL; US, vol. 37, no. 8, 1 October 2005 (2005-10-01), pages 3450-3451, XP027612451, ISSN: 0041-1345 [retrieved on 2005-10-01]

ATSUSHI FUJIO ET AL: "Collagenase H Is Crucial for Isolation of Rat Pancreatic Islets", CELL TRANSPLANTATION, vol. 23, no. 10, 17 October 2014 (2014-10-17), pages 1187-1198, XP055161407, ISSN: 0963-6897, DOI: 10.3727/096368913X668654

SALAMONE M ET AL: "A New Method to Value Efficiency of Enzyme Blends for Pancreatic Tissue Digestion", TRANSPLANTATION PROCEEDINGS, ELSEVIER INC, ORLANDO, FL; US, vol. 42, no. 6, 1 July 2010 (2010-07-01), pages 2043-2048, XP027209568, ISSN: 0041-1345 [retrieved on 2010-08-06]

BRANDHORST H. ET AL.: 'The Importance of Tryptic-like Activity in Purified Enzyme Blends for Efficient Islet Isolation' TRANSPLANTATION vol. 87, no. 3, 15 February 2009, pages 370 - 375, XP055269693

S. YOSHIDA ET AL: "The Influence of Collagen III Expression on the Efficiency of Cell Isolation With the Use of Collagenase H", TRANSPLANTATION PROCEEDINGS, vol. 46, no. 6, 1 July 2014 (2014-07-01), pages 1942-1944, XP055161406, ISSN: 0041-1345, DOI: 10.1016/j.transproceed.2014.06.007

Enclosed is a translation of the patent claims in Norwegian. Please note that as per the Norwegian Patents Acts, section 66i the patent will receive protection in Norway only as far as there is agreement between the translation and the language of the application/patent granted at the EPO. In matters concerning the validity of the patent, language of the application/patent granted at the EPO will be used as the basis for the decision. The patent documents published by the EPO are available through Espacenet (<http://worldwide.espacenet.com>) or via the search engine on our website here: <https://search.patentstyret.no/>

PATENTKRAV

1. Fremgangsmåte for å separere pankreas øyer fra et pankreasvev, ved
5 anvendelse av en nedbrytende enzymblanding, som fremstilles ved å tilsette
kollagenase H og kollagenase G avledet fra *Clostridium* sp. i en mengde som er
tilstrekkelig til å nedbryte kollagen I og/eller kollagen III og i samsvar med
sammensetningen av kollagen I og/eller kollagen III i pankreasvevet, til en
10 forutbestemt mengde av en nøytral protease og/eller en protease avledet
fra *Clostridium* sp.,
hvor kollagenase H nedbryter kollagen I og kollagen III, og
hvor vektforholdet (H/G) mellom kollagenase H og kollagenase G i den nedbrytende
enzymblandingen er 0,35 eller mer.
- 15 2. Fremgangsmåte ifølge krav 1, hvor den nøytrale proteasen er termolysin eller
en nøytral protease avledet fra *Clostridium* sp.
3. Fremgangsmåte ifølge krav 2, hvor den nedbrytende enzymblandingen
inneholder termolysin 0,3 mg/10 ml, kollagenase G 2 mg/10 ml og kollagenase H 1,1
20 mg/10 ml.
4. Fremgangsmåte ifølge krav 2, hvor den nedbrytende enzymblandingen
inneholder termolysin 0,3 mg/10 ml, kollagenase G 8,4 mg/10 ml og kollagenase H
2,9 mg/10 ml.
- 25 5. Fremgangsmåte ifølge krav 1, hvor den nedbrytende enzymblandingen
inneholder en nøytral protease avledet fra *Clostridium* sp., som den nøytrale
proteasen, og en protease som har en α -N-benzoyl-L-arginin-etyl ester-hydrogenklorid
(BAEE) nedbrytende aktivitet, som proteasen avledet fra *Clostridium* sp.