

himac

himac
centrifuges

Practical Applications of Biological Centrifugation



CAUTION:

- For safe and proper use of your machine, carefully read and follow instruction manual.

- All specifications are subject to change without advanced notice.
- Actual color may vary from the color of the photos on this catalogue, due to printing condition.
- Due to safety reason, installation environments, operating environments and conditions may be restricted.
- Unless specially mentioned, products and/or operation panel of the photos are standard specifications.
- This CF-RN centrifuge series is not a medical device.
- For further information, please contact us.

himac

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We change our brand, himac from Hitachi, effective on June 2018. And change our company name, "Koki Holdings Co., Ltd." from "Hitachi Koki Co., Ltd." on June 2018.

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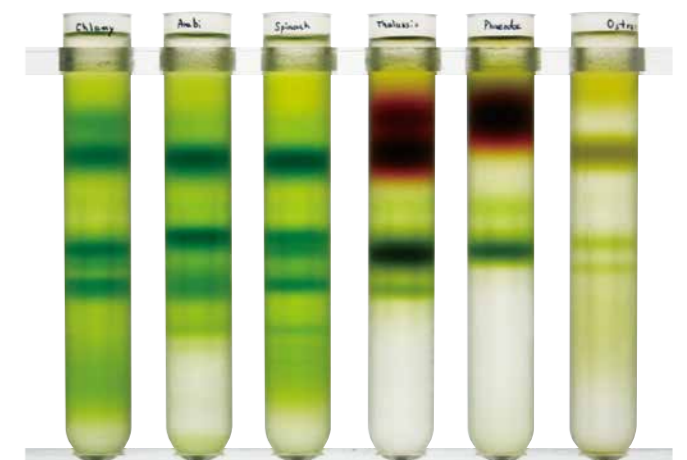


Photo credit: Dr. Tokutsu, NIBB

Acknowledgement: towards the publication of the Practical Applications of Biological Centrifugation

Thank you for your continued use of the himac centrifuges

After receiving a request from the Tokyo Institute of Technology Precision and Intelligence Laboratory and Hitachi Central Research Laboratory, this department was founded in 1952 to work on the development of an ultracentrifuge prototype. In year 2016, we celebrated the 60th anniversary since we had commercialized the Japan's first ultracentrifuge.

After World War II, Japanese scientists worked with great hope and ambitions towards the development of science and technology in Japan. At that time, we were also working on product development with aspirations to catch up and overtake foreign manufacturers.

Since that time, if we have been able to contribute to the advancement of science and technology in Japan as the one-and-only domestic manufacturer of ultracentrifuges, that would be our greatest honor.

In line with advancements in technology, the capabilities of centrifuges have advanced greatly over the past 60 years. Sixty years ago, the rpm limit of centrifuges was 40,000rpm, but the maximum global record is now 150,000 rpm¹⁾ The cooling method has changed from refrigeration to electric cooling, and the motor has changed from carbon brushes to inverters.

The uses of centrifuges have also changed. Currently, centrifuges are used for new line of processes such as exosome isolation, and applications in industrial areas such as carbon nano tubes or metallic nano particles are also increasing.

As a manufacturer, we believe that we are responsible for always understanding cutting-edge science, which changes rapidly over time, and to provide centrifuges that meets the new requirements. For this reason, we have asked many research scientists about their use of himac centrifuges "when and how" and compiled the contents of the interviews into this booklet.

We hope this will help the future students who will work for a bright future, as well as research scientists.

Finally, we sincerely wish to thank the 8 researchers who have kindly participated in this interview.

Organizer / Editor; Kohei Kakehashi, Koki Holdings Co., Ltd.

1) As of March 2016, comparison of micro ultracentrifuges by our company

C O N T E N T S

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* The Japanese first edition was published in June 2016 and the English contents are translated based on 2016's data.

Practical applications of centrifuges in the purification of photosynthetic protein complexes



National Institute for Basic Biology (NIBB)
Division of Environmental Photobiology (Jun Minagawa Lab)
Assistant professor Dr. Tokutsu Ryutaro (PhD. in Life sciences)
 2011 Graduate from Hokkaido University Graduate School (PhD.)
 2011 Research staff in NIBB
 2013 Assistant professor of the NIBB Division of Environmental Photobiology

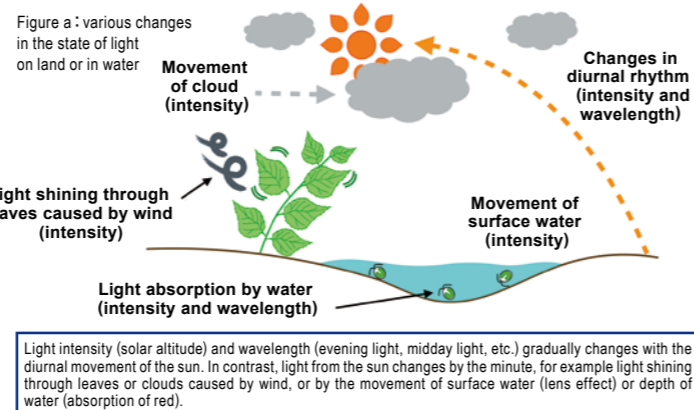
My research theme

Photosynthetic reactions have attracted the interests of many scientists since a long time, and its mechanisms have been studied. Conversely, it is still relatively unknown how photosynthetic organisms (plants or algae) adapt to the constantly-changing environment in nature.

For example, sunlight that reaches the earth's surface not only has seasonal or diurnal rhythms, but it may change dramatically in a short period of time (few seconds or minutes) due to changes in the states of clouds, winds, or water (Figure a). These organisms must adapt to the exact second that these environmental changes occur. In particular, sudden direct sunlight that may shine from in between leaves of trees or clouds may cause serious damage to photosynthetic organisms that were undergoing photosynthesis under low light intensity up to then. Such direct sunlight may provide excessive energy in an instant.

To avoid such danger, many photosynthetic organisms (plants or algae) are equipped with a mechanism called **non-photochemical quenching (NPQ) that eliminates light energy to avoid use in photosynthetic reactions only when the energy is available in excessive amounts**. NPQ is a mechanism that releases a part of excessive light energy by converting it into heat energy to avoid damage being caused to the photosynthetic pathway.

I have been studying the molecular mechanism of NPQ that is mentioned above. Up to now, I have mass-cultured green algae in the scale of several dozens of liters (bottom right photo), and purified photosynthetic protein complexes that are in a "live state" (photo on the right page). I revealed that NPQ occurs within the protein supercomplex called photosystem II. Currently, I am working on how NPQ occurs within the photosystem II protein super complex. Purification of a more complete protein supercomplex and their analysis are being conducted.



Lab scenery



There are more than ten himac centrifuges in the Minagawa lab including two ultracentrifuges and two high-speed refrigerated centrifuges.



Chlamydomonas
 A single-cell photosynthetic organisms, size approx. 10-20μm



To eliminate the effect of heat generated in association with light irradiation, culture is conducted in large-scale refrigerated tanks.

Reasons for using these centrifuges

Effective large-scale harvesting of Chlamydomonas using the R9A2 rotor (4×1500mL bottles)/ Purification of live photosynthetic proteins by sucrose density gradient

In our laboratory, we use the green algae Chlamydomonas as the model photosynthetic organism (central bottom photo on the left page). Chlamydomonas has a photosynthetic system that is similar to land plants, it is possible to mass-culture, and it can be easily genetically modified. Refrigerated centrifuges and ultracentrifuges are essential for harvesting mass-cultured Chlamydomonas and to purify photosynthetic protein complexes.

We were previously harvesting cells using middle capacity angle rotors. However, cultured cell-volumes needed to be minimized to fit the volume of the bottle for rapid harvesting that allows maintenance of the integrity of cells or proteins. Our studies are now performed more efficiently by using large-capacity 6 L rotor (R9A2).

Furthermore, ultracentrifuge swing rotors for 13 mL or 40 mL tubes, such as P28S and P40ST, are used to purify "live" photosynthetic complexes from various photosynthetic organisms, which are all unique to individual organisms.

Recommendation point of himac centrifuges

R9A2 Fixed Angle Rotor



Before centrifugation



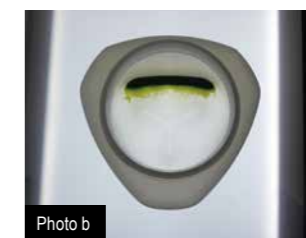
6,000rpm
 5min

After centrifugation



[The himac unique triangular-shaped 1500mL bottle]

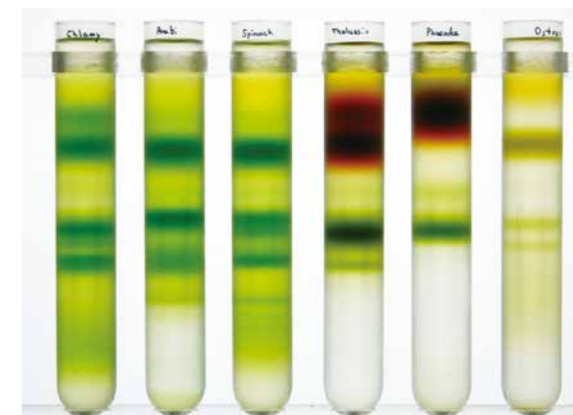
- "The volume per bottle is large; therefore, the total number of bottles can be minimized. This increases efficiency. The bottle is also triangular shaped; hence, the supernatant collects at the corner of the bottle while decanting. Everyone mentions it is easier to decant than using round cylindrical bottles"
- "It is rare for large-capacity bottles that it can be centrifuged with the desired volume of liquids. There is no problem in using empty bottles for centrifuging. This is a useful feature, for example, when you have a small volume of culture medium left."



a : 1.5 L bottle decanting
 b : 1.5 L bottle seen from above

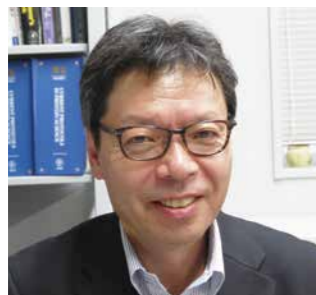


Thylakoid membranes isolated using the ultracentrifuge swinging bucket rotor is then dissolved, and are fractionated using density gradient centrifugation.



Unique protein complexes can be purified from various photosynthetic organisms by sucrose gradient centrifugation.

Practical applications of centrifuges in Protein structural analysis



RIKEN SPring-8 Center (RSC) Biometal Science Laboratory

Dr. Yoshitsugu Shiro (PhD., Engineering)

1986 Kyoto University Graduate School of Engineering and Faculty of Engineering, major in petrochemistry (PhD.)

1987 RIKEN, researcher

2000 Shiro biometal science laboratory, group leader

*as of March 2019, Professor of University of Hyogo School of Science, School of Science Graduate, School of Life Science, Cellular Regulation

My research theme

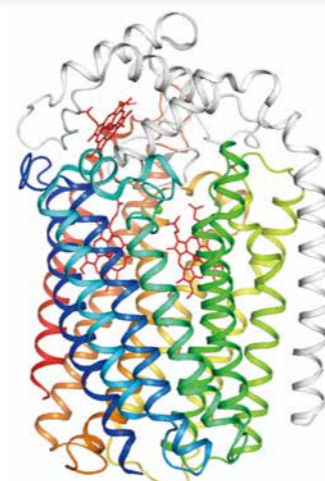
Understanding the mechanisms of chemical reactions via protein structural analysis

Three-dimensional (3D) structures of membrane proteins or enzymes containing iron, the element that is essential for maintaining life, are being studied in Shiro laboratory. From such structural information, their bioactivities and mechanisms of chemical reactions are further being studied. In future, we aim to develop technologies for visualizing the association or dissociation of protein complexes during biological active reactions at the molecular using the X-ray Free Electron Laser (XFEL) facility, such as SACLA that is built next to RIKEN (Harima).

Visualizing the courses of reactions with SPring-8

In general, structural analysis of membrane proteins has not advanced greatly because of the difficulties in obtaining high-quality crystals that are suitable for use in structural analysis. Dr. Shiro has succeeded in creating high-quality crystals by highly-purifying membrane proteins expressed in *Pseudomonas aeruginosa*.

SPring-8 is a large-scale synchrotron facility that allows analysis of microcrystals at high resolution. From X-ray crystallography analysis using SPring-8, the 3D structures of various proteins, such as nitric oxide reductase (NOR), have been elucidated.



Crystal and crystal structure of nitric oxide reductase (NOR)

Lab scenery



[Large-capacity rotors for harvesting bacteria]
We select the rotors depending on the samples and volumes.



[Bottles allocated for harvesting bacteria]
We prepare a number of bottles to speedily harvest bacteria.



The ultracentrifuge for purification is an essential tool in Shiro laboratory.

Reasons for using these centrifuges

For the membrane protein that we are studying, we are only able to obtain about 1 mg of the protein from 1000 mL of culture medium. We need to be able to mass-culture and purify the protein to make progress with research.

•[Harvesting] Effective mass-harvesting of *Pseudomonas aeruginosa* using large-capacity 1000 mL bottles.

We prepare 4 × 1000 mL bottles filled with culture medium while centrifuging a different set of 4 × 1000 mL bottles. When spinning is complete, the bottles are swapped for centrifuging. This is repeated to effectively harvest bacteria from 20 L of culture medium.



A large volume of *P. aeruginosa* is always harvested in Shiro lab, so 20 L of *P. aeruginosa* is cultured using 4 × 5 L flasks.



<Harvesting>
8,000rpm 10min 4°C
R9AF fixed angle rotor/
1000 mL bottle



Bottles after harvesting will be stored at -80°C

•[Purification by ultracentrifugation] Sucrose density gradient centrifugation of membrane proteins using middle capacity fixed angle rotors

The first step in purification of membrane fractions obtained in large volumes is sucrose density gradient centrifugation. To process a large volume in one round of centrifugation, we use P45AT that allows spinning of 6 × 70 mL bottles.



<Collection of the membrane fraction>
Sonicated *P. aeruginosa* is spun in an ultracentrifuge at 40,000 rpm for 1h at 4°C using the P45ATrotor/70 PC bottle. The supernatant is the soluble fraction (brown). The precipitate is the membrane fraction (reddish-purple).



After dissolving in surfactants, it is spun at 40,000 rpm for 12h at 4°C (sucrose density gradient centrifugation)

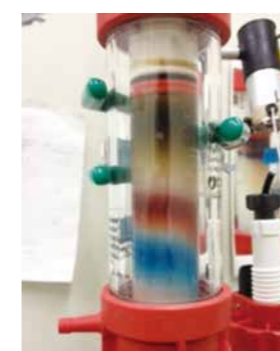


<Purification of membrane proteins>
Fractions are nicely separated using fixed angle rotors. The band containing the target protein is collected and purified. It is then crystallized!



P45AT angle rotor
6 × 70 mL bottles
MAX : 45,000rpm 235,000×g
Using convenient screw-cap bottles, medium volumes can be centrifuged at greater than 200,000 ×g.

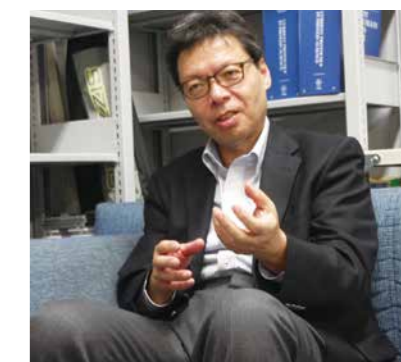
•[Anion-exchange column chromatography] Fractionation of soluble fractions



The soluble fractions are taken out separately and purified using anion-exchange column chromatography



Collected by the fraction collector.
Blue is protein containing copper.
Red is protein containing one iron.
Greenish-brown is protein containing four irons.



"The volume and purity is important in crystal structure analysis. We collect samples with the centrifuge, and carefully purify them."

Practical applications of centrifuges in the purification of rice organelles



Niigata University Faculty of Agriculture, Department of Applied Biological Chemistry (Professor Toshiaki Mitsui's Lab)

Specially Appointed Assistant Professor Kazusato Oikawa, (PhD. in Physiology)

2002-2008 Japan Society for the Promotion of Science Research Fellow

2004 Graduated from Tokyo Metropolitan University, Faculty and Graduate School of Science, Major in Biological Sciences

2008 National Institute for Basic Biology Division of Cell Mechanisms, researcher

2013 Niigata University Faculty of Agriculture, Department of Applied Biological Chemistry, Specially Appointed Assistant Professor

*as of March 2019, Research Scientist of RIKEN CSRS Biomacromolecules Research Team, Numata Laboratory

Our research theme

Rice is one of the most important crops for humans. The environment has been deteriorating over the years, and the rise in temperature and CO₂ levels are particularly concerning factors that cause environmental damage.

In our laboratory, we use methods in cell biology, physiology, and biochemistry to study ways for producing high-quality rice under various environmental stresses. In particular, we focus on organelles. We also study intracellular protein transport, stress-responses gene expression or organelle interaction mechanisms.



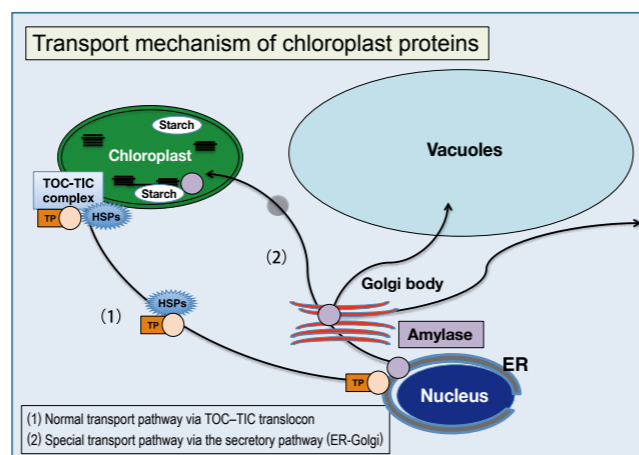
(Photo above) Niigata University Kariwa Village Advanced Agro-Biotechnology Research Center. Professor Mitsui from our lab is the head of this research center.

We now work on the cultivation of resistant rice to high-temperature/high-CO₂, and work on identifying mutants at the Niigata University Kariwa Village Advanced Agro-Biotechnology Research Center. We goal is to contribute to local areas through our results to revitalize agriculture and forestry, and protect the woods and costal environments.

(Left photo) Sado Island can be viewed from the lab on a clear day. Because of its location in proximity to rice agriculture, active research on rice is conducted at Niigata University. Rice is the staple food in Asia, and one of the important research subjects in Japan in academia and as a food.

My research theme Analysis of amylase transport mechanism which transported to plastid via secretory pathway

Proper degradation of starch in response to its surrounding environment or growth leads to supplying energy to various tissues by translocation, or storing in seeds. This is an important mechanism for the survival of plants. Amylase is one of the main proteins that is involved in starch degradation, and possessed by many organisms. In our lab, we showed that rice α-amylase is transported to chloroplasts via the secretory pathway (ER-Golgi body). We are now focusing on this chloroplast transport mechanism of amylase, and working on identifying related factors and their regulatory mechanisms.



Reasons for using these centrifuges

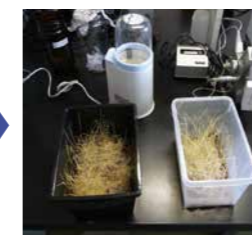
Efficient organelle isolation using two types of ultracentrifuge swinging bucket rotors

As a methodology in biochemistry, ultracentrifuge swinging bucket rotors are used to isolate organelles, such as Golgi body, chloroplasts, and mitochondria, from mutant/transformant rice or rice grown under various environmental conditions. Damage to these organelles is minimized during isolation.

First, to purify the whole extract several times and concentrate to target organelle fractions, swinging bucket rotor P32ST is used. P32ST is for 40mL tubes, which are largish volume in ultracentrifuge rotors. After purifying the total extract several times, target organelle fractions are concentrated. Next, density gradient centrifugation is performed using the swinging bucket rotor P40ST to gradually increase purity. Long and narrow 13 mL tubes are used in P40ST.



Many rice plants are cultured in the lab.



We use 10 cases of rice in one round of centrifugation.

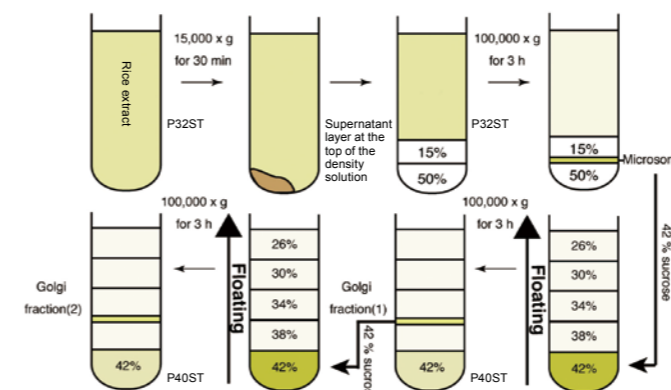


Rice is cut by scissors, which is hard work, but necessary for our research.

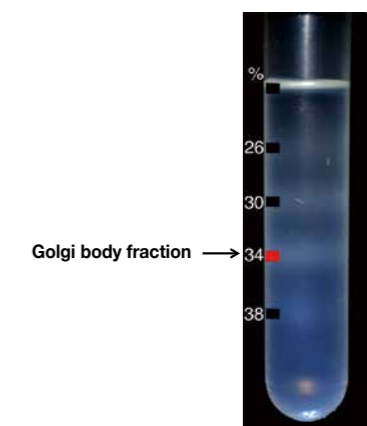


Now, insert tubes into the swing rotor! Purity increases with the use of the ultracentrifuge and the swing rotor!

Process of cell fractionation (Golgi body)



Sucrose density gradient centrifugation



[Top half: microsome purification process/P32ST swinging bucket rotor (40mL tube)]
[Bottom half: Golgi body purification process/P40ST swinging bucket rotor (13mL tube)]

Point!

By floating centrifugation twice using long and narrow 13 mL tubes, high purity Golgi body can be isolated from the microsome, and minor contaminations, such as mitochondria, can be removed.

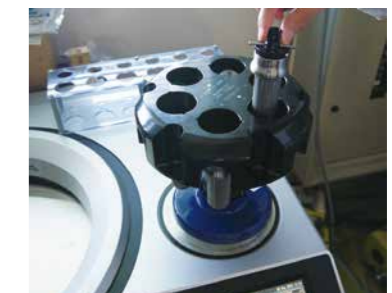
Recommendation points about himac centrifuges



A safety lecture on the use of centrifuges was conducted in the lab. A talk was given on the basics so that the centrifuges can be safely used by students.



The number of runs and total hours run are automatically calculated per rotor by the ultracentrifuge (CP-NX series). Its lifetime will be managed automatically. Daily recordings by staff will become unnecessary, which saves time for lab members



P32ST swinging bucket rotor for 40 mL tubes is easy to set, as the buckets are inserted from above!

Practical applications of centrifuges: Structural analysis of nerve cell membrane proteins



The University of Tokyo Synchrotron Radiation Research Organization (SRRO),
Life Science Department (Associate Professor Shuya Fukai Lab)

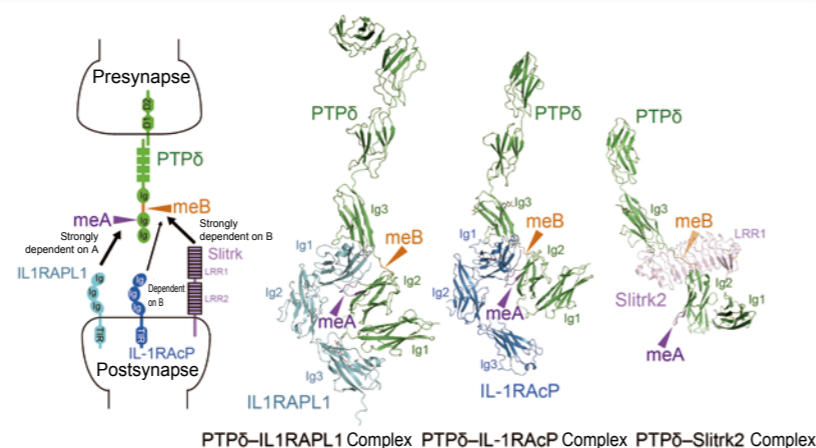
Assistant Professor Atsushi Yamagata, (PhD. in Science)

2002 Osaka University, Graduate School of Science and Faculty of Science, Major in Biological Sciences
2002 Japan Society for the Promotion of Science PD
2003 Researcher at The Scripps Research Institute, Department of Molecular Biology
2007 The University of Tokyo SRRO, Life Science Department, Associate Professor
*as of March 2019, Assistant Professor of Research Division for Quantitative Life Sciences, Institute for Quantitative Biosciences, the University of Tokyo

My research theme

Our brains consist of a complex network formed of several hundred billion neuron. The neuron are connected with each other through an adhesive structure called synapses. Synaptic formation is induced when transmembrane receptors called synapse organizers that exist on the pre-synapse and post-synapse form complexes. However, only about 10 types of synapse organizers are known to date, and it is largely unknown how such limited number of synapse organizers determine the diversity and specificity of synaptic junctions that occur among the several hundred billion neuron in the brain. We are trying to answer this question by understanding the diversity and specificity of synaptic junctions through structural analysis of synapse organizer complexes.

Recently, we conducted a structural analysis of the receptor-type tyrosine phosphatase PTP δ , which is one of the most well-known presynapse organizers, together with the various postsynaptic organizers that bind to it. These structural analyses revealed that the different structures of two peptide insertion sites (meA and meB) in PTP δ as a result of alternative splicing was an important element to the individual postsynapse organizers.



Lab scenery



We use a number of rotors, such as the large-capacity rotor for bacterial harvesting and rotors for 50 mL centrifuge tubes in high speed refrigerated centrifuges.



These are high-speed micro centrifuges, and the equipment at the very end is the micro ultracentrifuge

Reasons for using these centrifuges

We currently purify solubility proteins, intramembrane proteins or extracellular proteins using three expression systems: *E.coli*, insect cells and mammalian cultured cells.

Recommendation points about himac centrifuges

[Ultracentrifuge]

Collection and analysis of GFP-fused membrane proteins characteristics using P50A3 rotor (24×1.5 mL tube)



P50A3 fixed angle rotor 24×1.5 mL tubes, MAX: 50,000rpm, 252,000×g.

The himac original 1.5 mL microtubes (right photo) can use up to 252,000×g in ultracentrifuges. Further, 24 tubes can be spun at once!

"Handling of ultracentrifuge tubes may seem difficult, but it can be easily operated when we use the familiar microtubes. It is a useful rotor that allows us to purify samples of very small volumes."



Ultracentrifuge for 30 min
at 40,000 rpm

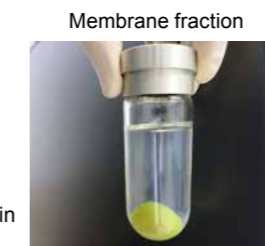


Photo: 70PC bottle (of P45AT rotor)



Add 1% DDM, and
solubilization with a
homogenizer and stirrer.



Photo: 70PC bottle (of P45AT rotor)

[High-speed refrigerated centrifuges]

Harvesting of *E.coli* for mass-expression or collection from mammalian cell culture containing extracellular domains of receptors using the high-capacity R9A fixed angle rotor (4×1000 mL bottle).



R9A fixed angle rotor 4×1000mL bottles; MAX: 9000rpm, 15,300×g

himac original 1000 mL PP bottle (Wide Mouth)

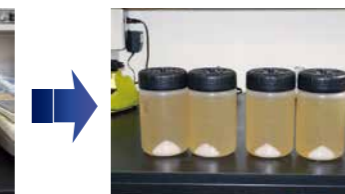
Characteristic 1: One can use the desired volume. There is no problem even if you spin empty bottles.

Most centrifuge rotors normally require filling of large-capacity bottles up to at least 80% of its capacity. Do you spin them full every time? Do you dilute the liquid volume to fill the bottle, ignore the little bit of medium left, or adjust the culture volume?

"While this is a large-capacity rotor, but it is also applicable for medium-volumes."

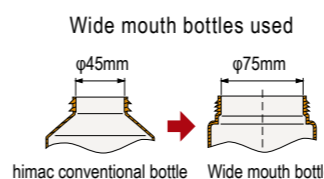
Characteristic 2: Increased efficiency of pellet collection with wide-mouth bottles (left figure)

Have you ever been irritated by narrow-mouth bottles during collection or cleaning?
"Harvesting of *E.coli* is routine work; therefore, we select bottles that are easy to handle."

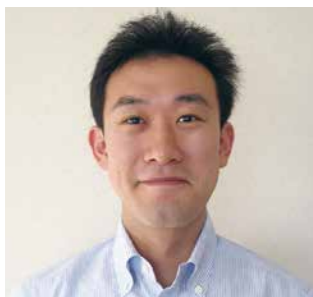


7,000×g
10min

"You can spin the desired volume even if you have a small volume of culture medium left"



Practical applications of centrifuges in Exosome Isolation



Nagoya University Graduate School of Engineering, Department of Biomolecular Engineering (Prof. Yoshinori Baba Lab)

Assistant Professor, Dr. Takao Yasui, (PhD. in Engineering)

April 2009 – Nov 2011 Japan Society for the Promotion of Science DC1
 Nov 2011 Graduated from Nagoya University Graduate School of Engineering, Department of Biomolecular Engineering (PhD. Engineering)
 Nov 2011 Japan Society for the Promotion of Science PD
 Jan 2012 Nagoya University Graduate School of Engineering, Assistant Professor
 Nov 2014 Impulsing Paradigm Change through Disruptive Technologies Program (ImPACT) Assistant PM
 Oct 2015 JST PRESTO (Sakigake) researcher
 *As of March 2019, Associated professor of Graduate School of Engineering Biomolecular Engineering, Nagoya University

My research theme

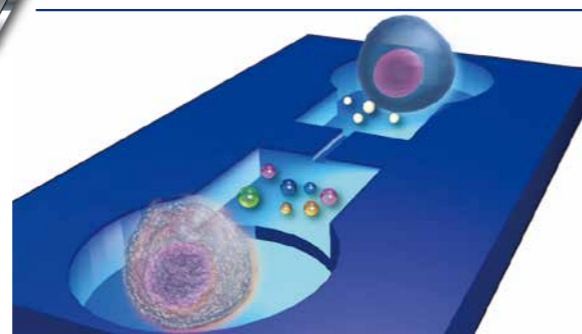
Making exosome analysis possible in a nano space

Exosomes are 40-200 nm endosome-derived small vacuoles with lipid bilayer membranes that are secreted by cells. MicroRNAs that function as regulators of biological processes were recently discovered to exist in exosomes. Cancer cell-derived exosomes have gained attention as a potential oncogenic factor. We have created a nano space that allows us to perform quantitative analysis of exosomes, which is an oncogenic factor that is transported from cancer cells to normal cells.

Reasons for using these centrifuges

Development of an exosome isolation technique using nano space that does not require centrifugation or commercial kits

In Baba lab that Dr. Yasui is affiliated with, a unique method of efficiently extracting exosome-derived MicroRNAs is being studied. In their method, exosomes are adsorbed within a nano space created within the microchannel from a small volume of body fluid or culture supernatants. Currently, Dr. Yasui works on selectively capturing exosomes that are released from cancer cells in nano space. During sample preparation, a micro ultracentrifuge is used for exosome collection. Their main study theme includes determining what type of exosomes can be collected by changing the shape, layout patterns, and surface materials of the nano spaces.



Selective isolation of exosomes in nano spaces

By passing exosomes released from cancer cells through a nano space, the goal is to selectively isolate target exosomes.

Recommendation points about himac centrifuges

"We use the micro ultracentrifuge in our lab. It is one size smaller than normal ultracentrifuges and can be placed anywhere in the lab. As it also operates under 100 V power supply, it can be placed right next to the clean bench for exosome collection."



S50A fixed angle rotor rotor
 (for micro ultracentrifuges)
 Max capacity: 6×30.0mL
 MAX : 50,000rpm 210,000×g
Efficient collection of exosomes using the large-capacity fixed angle rotor in micro ultracentrifuge.

Point!

Up to 30.0 mL tubes can be used in himac micro ultracentrifuges. Although a general ultracentrifuge was initially used in the lab, the micro ultracentrifuge was selected because it can be placed anywhere in the lab, and because it is easy to use.



Thick-walled tubes that do not require lids that can be used with any volume, or tubes with screw-type lids are often used.



110,000×g, 80min exosome fraction. The sediment can be seen by the naked eye.

Recommendation from himac centrifuges: Micro ultracentrifuge series



CS150NX
(Benchtop)



CS150FNX
(Floor-standing)

In recent years, more customers select micro ultracentrifuges. Prices and sizes are suitable for purchase by single laboratories. We will introduce the micro ultracentrifuge used by Dr. Yasui.

- (1) **The space requirements**
 They are one size smaller than normal ultra-centrifuges and operate on 100 V electric supply. Time-consuming electrical work is not needed. It is popular because it can be placed in a small space that is available in the lab.
- (2) **Rotor Line-up**
- (3) **Easy-to-handle thick-walled tubes that can be used with the desired volume without lids**
 Thick-walled tubes for 1–25 ml volumes are available for himac micro ultracentrifuge rotors. As there are no lids, and a desired volume can be used; it is easy to handle.

[We recommend the floor-standing type micro ultracentrifuge]

The micro ultracentrifuges are popular due to their compact size.

You may choose either the benchtop type or floor-standing type from himac micro ultracentrifuges.

Most Japanese users appear to prefer the floor-standing type for the following reasons (comparison of company products).

- (1) It may be hard to directly see the axis of tabletop types depending on the height of the lab bench, and there is a risk of mistakes in placement.

You can smoothly insert the rotor, even for unstable liquid surfaces, such as during density gradient centrifugation, if you use the floor-standing type.



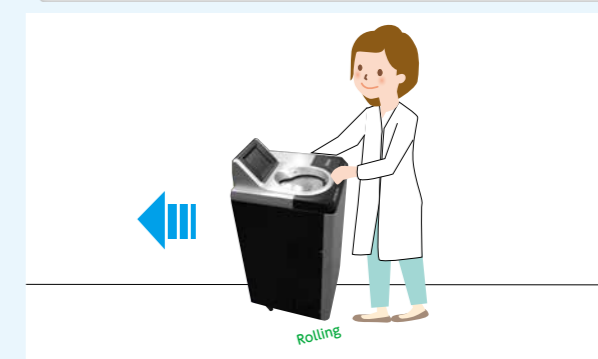
- (3) The footprint of floor standing type is small (comparison between our company products).



The floor-standing type can be placed anywhere, and will not take up the entire bench!

- (2) The floor-standing type has wheels, and it is easy to move.

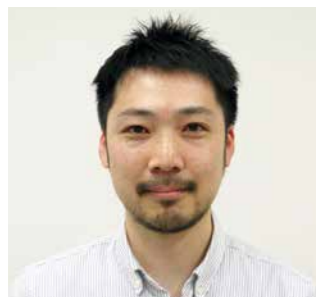
Centrifuges are for long-term use. It may be moved to a different research site or may be moved within the lab over a short distance.



- (4) The benchtop type will be more spacious while working.



Practical applications of centrifuges in Identifying novel enzymatic proteins



Asano Active Enzyme Molecule Project[※]

Toyama Prefectural University,
Biotechnology Research Center (Prof. Yasuhisa Asano Lab.)

Dr. Takuya Yamaguchi (PhD. in Agriculture)

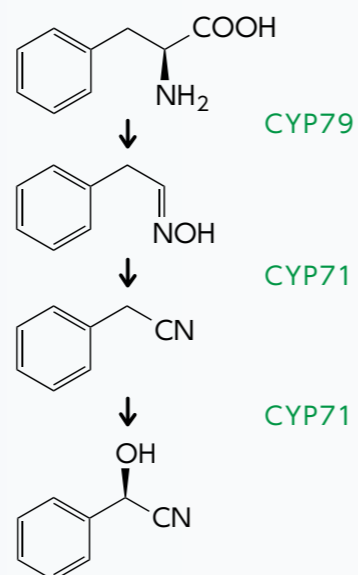
2012 Graduated from Hokkaido University, Graduate School of Agriculture, Division of Agrobiolgy, PhD
2012 Toyama Prefectural University ERATO[※]Asano Active Enzyme Molecule Project. Researcher
[※]ERATO: Japan Science and Technology Agency, Strategic Basic Research Programs, ERATO.
2015 Above project, Biological resources group, Group leader
*As of March 2019, Assistant professor of Faculty of Life and Environmental Sciences, University of Tsukuba

Project summary

Enzymes are proteins produced by living organisms that efficiently catalyze chemical reactions under mild conditions. Enzyme-applied technology is called white biotechnology and gaining attention for being one of the technologies that are eco-friendly within the chemical industry. While industrial enzymes have been discovered mainly from microorganisms, we focus on plants and animals as sources of industrial enzymes in this project and look for novel proteins from these sources.

We are also working on elucidating the various properties related to the chemistry of novel enzymes from plants and animals, and develop methods utilizing these enzymes. We hope to contribute to the development of white biotechnology in the future through these activities.

In some plants, nitrile compounds with chemical defense properties are biosynthesized from amino acids. We discovered cytochrome P450 (CYP79 and CYP71) involved in their biosynthesis from *Prunus mume* or *Fallopia sachalinensis*. As nitrile compounds are useful industrially, we believe that plant-derived cytochrome P450 can be used to make nitrile compounds by fermentation. We are therefore working on understanding the various properties related to enzyme chemistry of cytochrome P450, as well as construction of mass-expression systems.

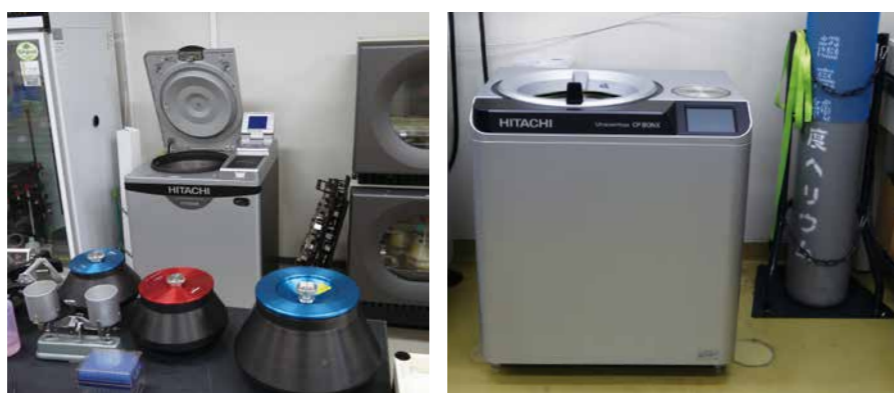


Lab scenery

Ultracentrifuge
(Right photo: CP80NX)

High-speed refrigerated
centrifuge
(Left photo: CR22GIII)

Many himac centrifuges such
as the ones shown are used.



Reasons for using these centrifuges

Efficient harvesting and removal of membrane fractions using the ultracentrifuge large volume fixed angle rotor P27A (6×160mL bottles, MAX 106,000×g)

The plant cytochrome P450 is a membrane-bound protein. To study the various properties related to enzyme chemistry of cytochrome P450, membrane fractions must be collected or removed from a large volume of samples.

In the past, we needed to perform ultracentrifugation several times, but almost 1 L of sample can be processed at 100,000 ×g in one centrifugation using the himac original large volume ultracentrifuge rotors, which allows us to save time on sample preparation.

Recommended points about himac centrifuges



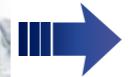
Bulk culture of *E. coli*



After bacterial harvesting, the solution turns red when cytochrome P450 is extracted by ultrasonication.



P27A fixed angle rotor
(6×160mL bottles, MAX 106,000×g)
A minimum of 100,000 X g is required
even using a large volume rotor
when spinning biological samples



Before centrifugation



After centrifugation

27,000rpm
106,000×g, 60min

Practical applications of centrifuges in the experimental infection in cells



Keio University, School of Medicine, Institute for Advanced Medical Research, Division of Gene Regulation (Prof. Hideyuki Saya Lab)

Specially Appointed Assistant Professor, Dr. Nobuyuki Onishi (PhD. in Medicine)

April 2005 Japan Society for the Promotion of Science DC1

2008 Graduate of Kobe University, Graduate School of Medicine, PhD. (M.D.)

April 2008 Kobe University, Graduate School of Medicine, Researcher

Oct 2008 Keio University, School of Medicine, Specially Appointed Assistant Professor

* As of March 2019, Project Instructor of Division of Gene Regulation, Institute for Advanced Medical Research, School of Medicine, Keio University and Researcher of Technology Research Laboratory, Life Science Research Center, Shimadzu Corporation.

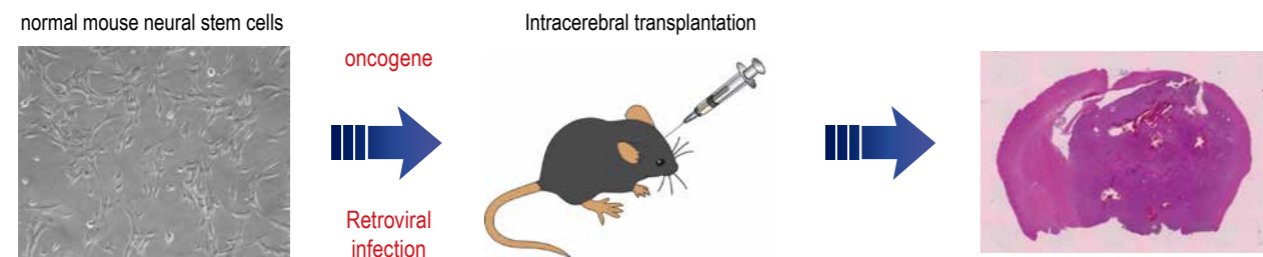
My research theme

Malignant brain tumors, particularly glioblastoma multiforme (GBM), are the most malignant out of all primary brain tumors. Removal by surgery is difficult due to its rapid infiltration. Because of its resistance to radiation therapy and chemotherapy, prognosis is poor. It is essential to construct appropriate carcinogenic models in order to developing new treatment strategies by understanding fully the characteristics of GBM.

Previously, we have constructed a mouse brain tumor model with characteristics similar to human GBM. This was done by introducing oncogenes into normal mouse neural stem cells using retroviruses, which were transplanted into the same strain of the mouse brain. Furthermore, oncogenes were inserted into the transposon sequences of normal mouse neural stem cells using the piggyBac system, which lead to the successful establishment of induced Cancer Stem Cells (iCSCs) that form tumors within the mouse brain. Furthermore, we are developing simple, stable novel brain tumor models by directly introducing cancer transgenes into the mouse brain to replicate the human carcinogenic process that is closer to the real clinical situation.

By performing detailed analyses using these brain tumor models, we are hoping to reveal the molecular basis of carcinogenesis in brain tumors and the process of increased malignancy.

[Construction of a brain tumor model by retroviral infection using the Ex vivo system]



[Construction of a brain tumor model by direct introduction of oncogenes into mouse brain using the piggyBac system]



Reasons for using these centrifuges

Reducing required time for virus concentration using the himac 50 mL conical tubes (max centrifuge speed: 42,200 ×g)

To efficiently infect normal neural stem cells with retroviruses, it is essential to perform buffer exchange and centrifugal concentration of retroviruses in the supernatant of packaging cells culture medium to increase viral titer. A minimum of 3 hours was required for centrifugation using conventional high-speed refrigerated centrifuges (MAX: 15,000 rpm/30,190 × g) when we studied optimal conditions. **It became possible to concentrate sufficient volumes of retroviruses within 1 hour** by using himac high-speed refrigerated centrifuges (R18A fixed angle rotor).

Recommendation points about himac centrifuges



[R18A fixed angle rotor]
MAX : 18,000rpm/42,200 ×g
8 × 50 mL conical tubes



himac original 50 mL conical tubes for R18A (centrifugation at high ×g)

- (1) Can be used up to max. 42,200 ×g [actual capacity, 45 mL/tube]
- (2) Has a v-shaped bottom, so sediments collect more easily at the bottom of the tube compared to rounded-bottom tubes.
- (3) Has an air-tight screw top. Improved safety!
- (4) Radiation Sterilized
- (5) Volume scales and space for labels

Point!

A high-speed refrigerated centrifuge (angle rotor) can be used instead of ultracentrifuges (swing rotor), which had been used in collecting sediments that are hard to see by the eye, such as concentrated viruses. It is also cost effective. It is favored as "it is easier to use than the conical tubes that we have used up to now".



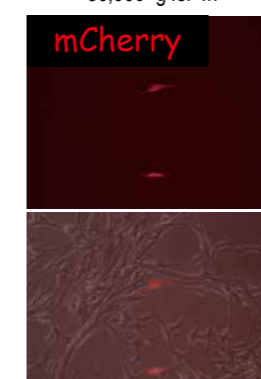
Culture supernatant of packaging cells using serum-free medium was centrifuged for 1 hour at 42,200 × g using a R18A fixed angle rotor. We obtained a visible white precipitate that was thought to contain retroviruses.

In considering the timing of preparing cells before infecting, it was very convenient to be able to reduce the time required for viral concentration to 1 h.

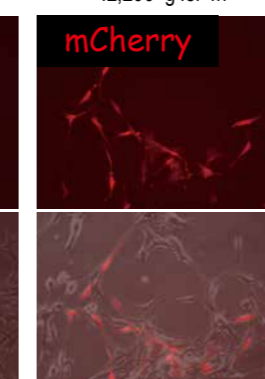
mCherry expression vector



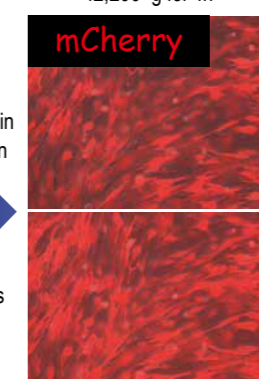
Conventional centrifugation
30,000×g for 1h



R18A rotor
42,200×g for 1h



R18A rotor
42,200×g for 1h



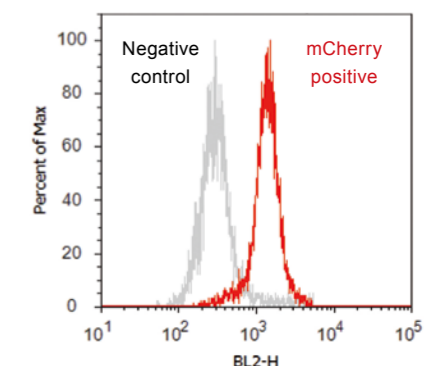
Puromycin selection

2 weeks

Confirmed expression of mCherry 3 days after retroviral infection

On confirming mCherry expression 3 days after retroviral infection, 5–10 times more infection was detected while using the himac high-speed refrigerated centrifuge in comparison with a conventional refrigerated centrifuge.

After 2 more weeks, approximately 100% mCherry positive normal neural stem cells were established by puromycin selection.



Detection of fluorescence intensity of mCherry using a flow cytometer
Vertical axis: relative cell count
Horizontal axis: fluorescence intensity

Practical applications of centrifuges in Regenerative Medicine



iPS PORTAL, Inc.
Research & Services Division

Assistant manager Dr. Seiji Hori (PhD. in Medicine)

1988 Completed Master's Program in Kyoto University Graduate School of Science
1994 Kyoto University PhD. in Medicine
Worked for Taiyo Gyogyo Central Laboratory (Current Maruha Nichiro Corporation), Novartis Pharmaceuticals, Osaka Bioscience Institute researcher, Lecturer at Kyoto University, and current position since 2014
* As of March 2019, Executive Officer, iPS PORTAL, Inc.



Company Overview

Regenerative medicine is an industry that is expected to exceed a market size of 1 trillion yen globally by 2020. iPS PORTAL, Inc. was founded as a company that took over the sales and technical support services of iPS Academia Japan, Inc., which is the patent management company for Kyoto University's iPS cells. Products handled include iPS-derived differentiated cells and reagents. In addition, the company provides technical support for cell culture, etc. iPS PORTAL is also funded by Kyoto prefecture, and certified as a company that falls under the stipulations for production deregulation of blood-derived products (National Strategic Special Zone).

The company strives to help industrial application of academic research results related to iPS cells. For example, the company creates or cultures iPS cells for companies, provides differentiated cells, or helps with resource constraints by training technicians who can perform iPS cell culture. We also participate in collaborative efforts in the development of iPS-related equipment. Our mission is to link the scientists specializing in regenerative medicine, companies interested in business development in this field, and manufacturers that develops regenerative medicine-related equipment or devices. iPS PORTAL also aims to become a portal company that covers a wide area of iPS-related businesses.

In the company's laboratory, cells differentiated from iPS cells or iPS cell related equipment can be seen or be tested. Company-associated scientists from other companies can use the facility for training or as part of their actual development. Research scientists and technicians visit from all over the country. The himac swinging bucket rotor for high-speed micro centrifuge CF16RN is used not only by scientists, but also for cell culture training of beginners and project managers. The swinging bucket rotor is used for isolating cells after trypsin treatment, or for harvesting iPS cells that are induced to differentiate. The fixed angle rotor is also used for sampling of collected RNA, or for sample processing in flow cytometry. Both rotors can be used in a single CF16RN centrifuge, so it is very convenient.



Many research scientists, project managers, and business owners in Japan visit the Interactive Lab of iPS PORTAL, Inc. They use the actual equipment in training and learn the techniques related to iPS cells. The right most machine is the himac high-speed micro centrifuge.



As a company that provides a bridge to iPS-related business, iPS PORTAL, Inc. hosts the iPS Cell Business Conference six times a year to help companies with unique technologies to start up business related to regenerative medicine. Many companies with membership gather and actively exchange information and knowledge.

Visit our website

<https://www.himac-science.com/>

himac Ultracentrifuges with over 60 years of experience



CAUTION:

- Pictures that are different from our recommended environment are included. Please read "Instruction Manual" carefully before using the product in order to use it correctly and safely.
- Please use it safely according to the items such as ⚠ "DANGER", ⚠ "WARNING", ⚠ "CAUTION" displayed on the instruction manual and product.
- When separating samples such as toxic substances, radioactive substances, pathogenic substances or blood which cannot be denied infectiousness, please take necessary safety measures.
- Do not use flammable or explosive samples or actively reactive substances. himac Centrifuges are not explosion-proof construction. Also, do not handle or place such substances near the equipment.
- Refer to the "Chemical resistance list" attached to the rotor, and do not use samples that are unusable for the material of the rotor (including bucket). It may cause corrosion of the rotor (including bucket).
- For any unclear points about the sample to be used, please contact the nearest sales agent.