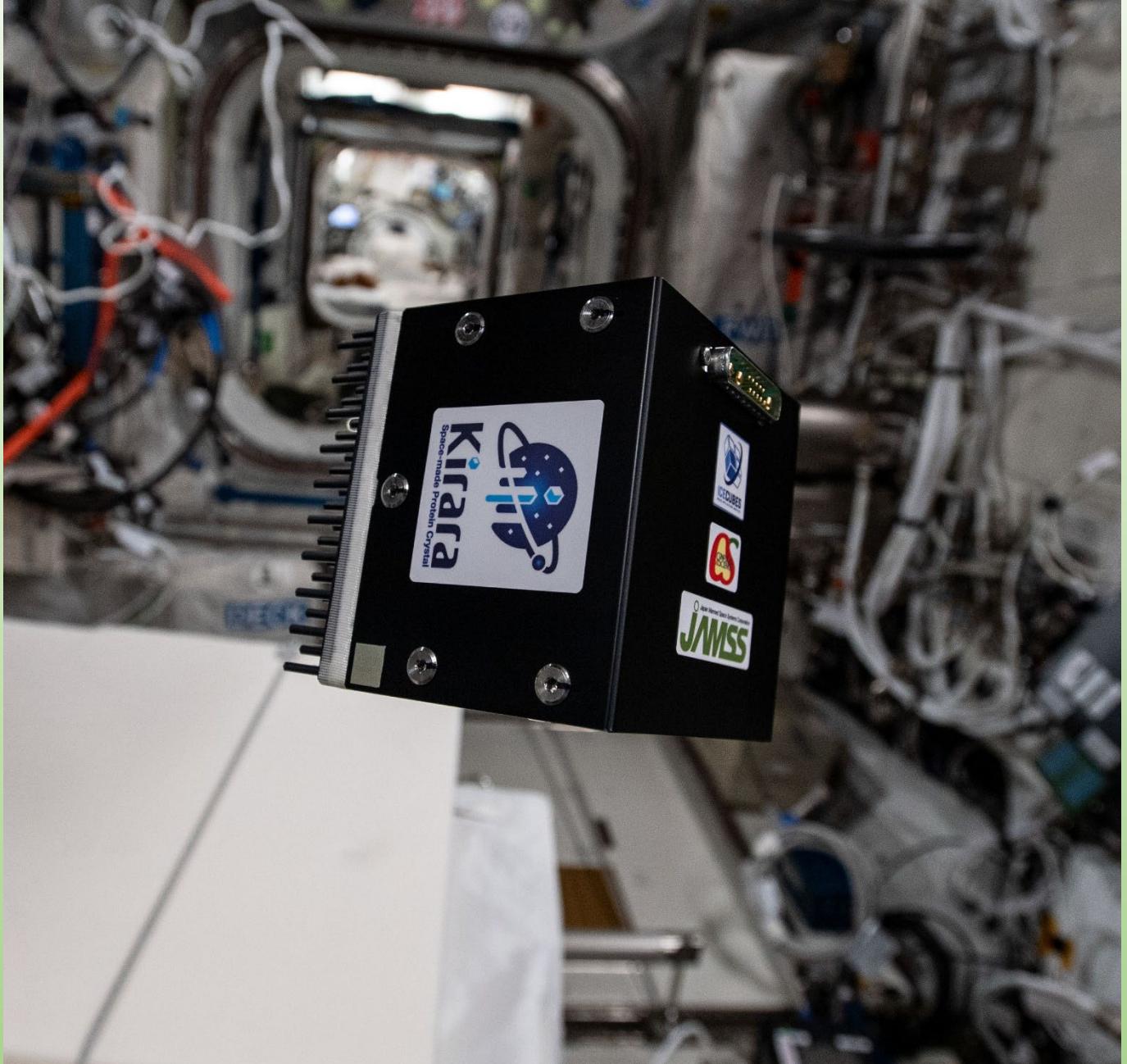


JMB-19K055C



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Kirara Application Guidelines

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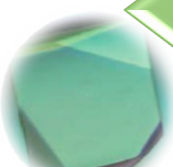


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Information Guide

High Quality Protein Crystal Growth Service “Kirara”

In many cases the microgravity environment of space, which it is not affected by convection or sedimentation, is beneficial to growing protein crystals of larger size and higher resolution than possible on the ground.

“Kirara” is a high-quality protein crystal growth service supporting drug discovery through the growth of high-quality protein crystals in space.

“Kirara” is also used for more than protein crystallization (i.e., Cellulose synthesis by enzyme).

One tube is 5.7 cm in length and has a volume of 1 mL. A capillary containing a protein sample is installed. Tubes are located in the incubator of 10cm cubic.



Crystallization tubes



Incubator

The JAMSS Cube holds up to 96 crystallization tubes, allowing multiple protein crystallization conditions to be used (as well as multiple proteins). The JAMSS Cube is set to **a temperature of 20 degree Celsius**.

We use a pipelined process that minimizes customers' paperwork to get your proteins research project ready for space. The primary paperwork for you is completing the Kirara application data sheets. Based on that we will take care of making various documents needed for the space mission.

After launch, protein crystallization is performed in space for about one month, after which the crystals will be returned to your facility.

The following delivery options are available:

1. Crystallization tube (crystals will need to be extracted from the tubes)
2. Frozen crystal (fixed on pins for X-ray diffraction) **as Default**
3. X-ray diffraction data (optional)

All deliverables and intellectual property rights obtained from this service belong to the customer.

Information Guide

Documents

1. Kirara order form for entry

Please fill in the order form of “Kirara” and send it to the following e-mail address.

If you need a confidentiality agreement in advance, please contact us for arrangement.

After submission of the order form, you can not add the composition of protein sample and various solutions. **Please list all possible reagents.** You can cancel them later.

For safety reasons, toxic and pathogenic samples cannot be sent to the space station. Make sure that the target protein, its raw materials and the manufacturing process are safe.

Please also check that your samples are not subject to import / export restrictions of U.S. as well as Japan.

2. Contract documents

We will conclude a contract before your sending us protein samples and crystallization solution. Please confirm the details of the implementation details, warranty scope, intellectual property rights, etc. in the contract.

Submission

Please send it to the following e-mail address, attaching the “Kirara” application data sheet that describes the necessary items.

E-mail: jamss-kirara@jamss.co.jp
Subject: Kirara Service application



*Please send only the order form to the address above.

We will contact you for detailed information of your samples after accepting your application.

Information Guide

Disclosure of Stated Information

In carrying out this service, the portion of the information * received from customers will be disclosed to the US NASA / Europe ESA for safety review of the International Space Station and the carrier company for safety judgment and custom clearance in a limited manner.

Other information will not be disclosed to third parties without your consent.

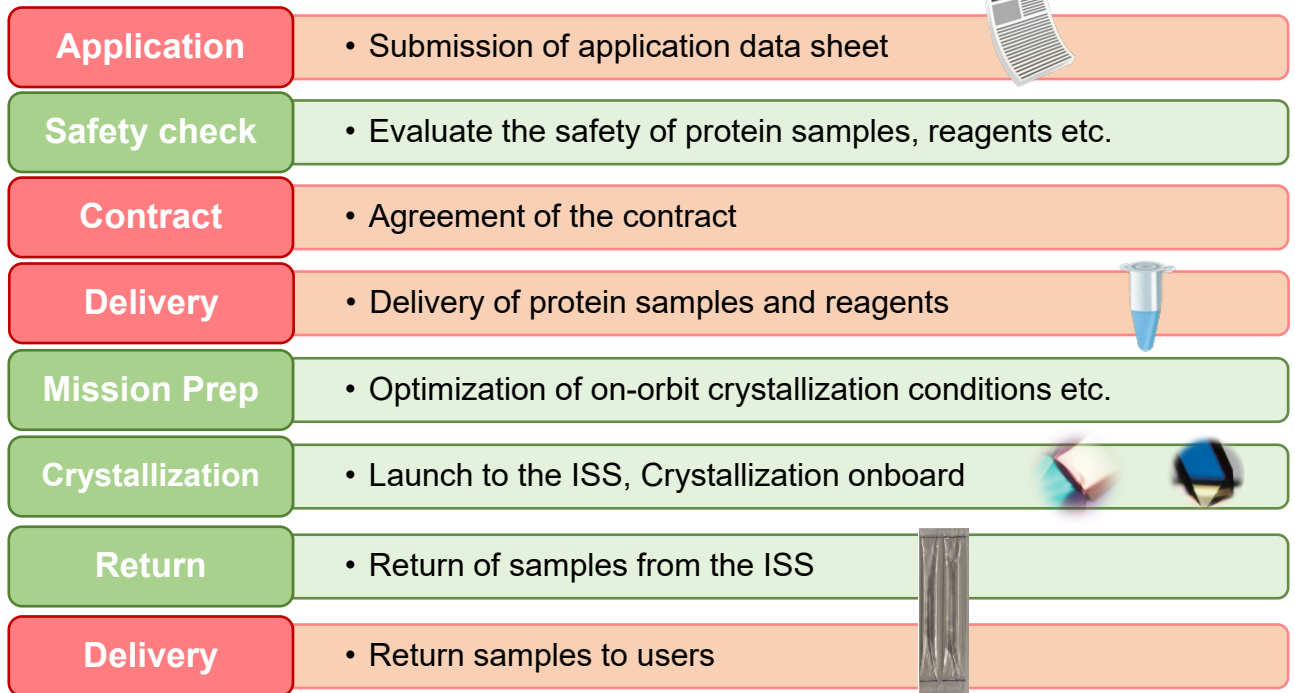
The following information will be disclosed to the US NASA / Europe ESA and the carrier.

- Identification of Protein (It can be arbitrary for confidentiality)
- Maximum concentration of protein sample
- Biological function of protein
- Origin of protein, Expression system
- Composition of crystallization solution, maximum concentration
- Safety Data Sheet (SDS) of the solutions

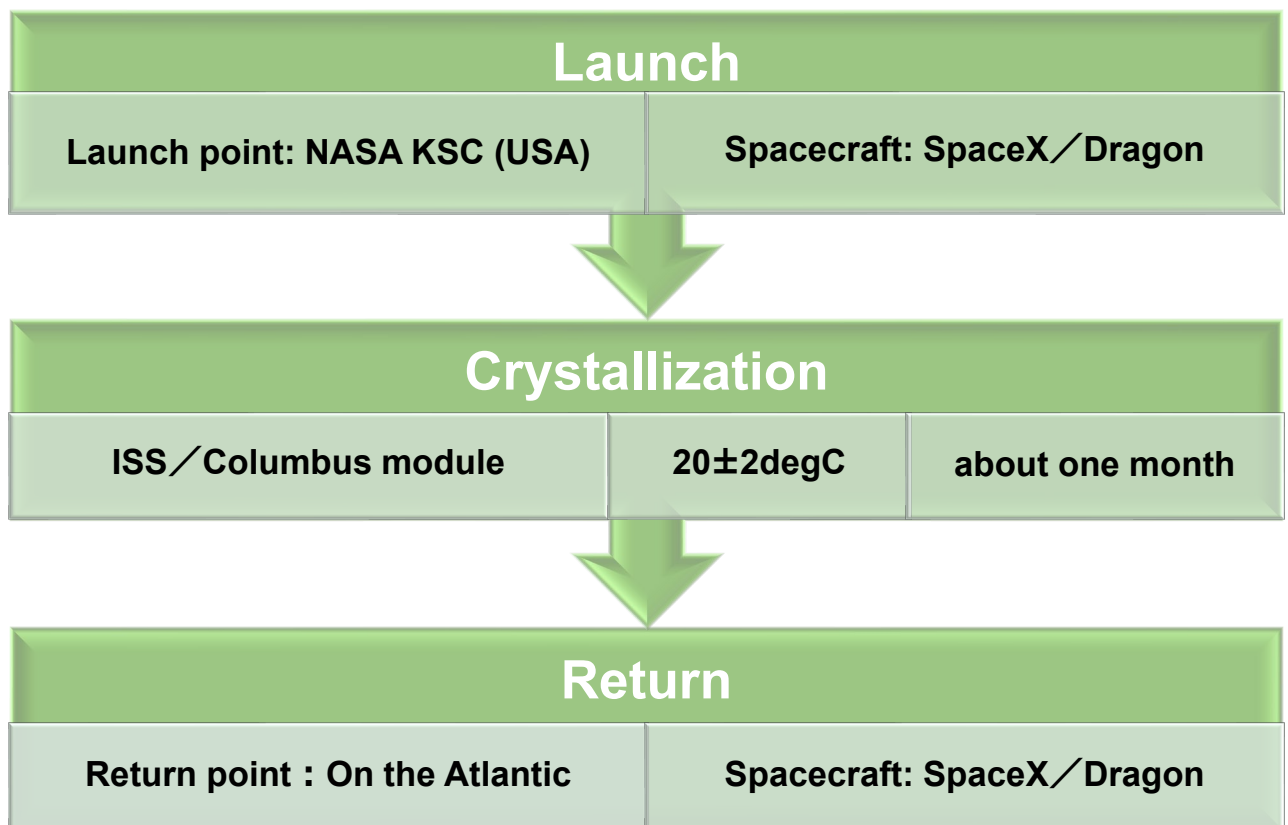
* The portion of the information is the information in the submitted "Kirara Order form". In the event that disclosure of information beyond that stated in the data sheet is required in the safety reviews, we will consult with customers regarding the scope and availability of disclosure in advance.

Implementation

Kirara Service Flow



Crystallization Onboard



Implementation

Sample Preparation and Delivery

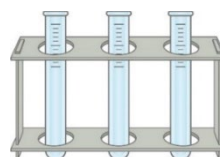
After applying, please send the protein sample and solutions. Transportation is **responsible to the customers** (including customs clearance if it is from overseas)

The crystallization conditions will be optimized using the protein sample which you sent.

solution	Amount*	Notes
Protein solution	50μl or more	Crystallization concentration
Crystallization solution	10ml or more	
Buffer solution	10ml or more	A solution obtained by removing the precipitant from the crystallization solution

* For one crystallization tube. If you crystallize the same protein with multiple compounds or use multiple crystallization tubes under the same conditions, you can reduce the amount of protein solution to be sent. It is about 20 μl for each additional crystallization tube. Please contact us for details. When freezing to send, to maintain sample quality, we recommend dividing the sample into two tubes: one for crystallization condition optimization (30 μl or more) and the other for space experiments (25 μl or more). Please note that to maintain quality, please prepare at least 25 μl of liquid per tube.

If you wish to get the frozen crystals or the X-ray diffraction data but the solutions are not proved freezing resistant, we may ask you to send solutions with cryoprotectant before freezing the crystals.



Optimization of Crystal Condition

With referring the crystallization conditions described in the application data sheet, we will optimize the conditions suitable for on-orbit crystal formation.

For optimization, we will use some of the protein samples and crystallization solutions received. The implementation period is about one month.

The optimized crystallization conditions will be informed the customers later.

Implementation

Crystallization in Space

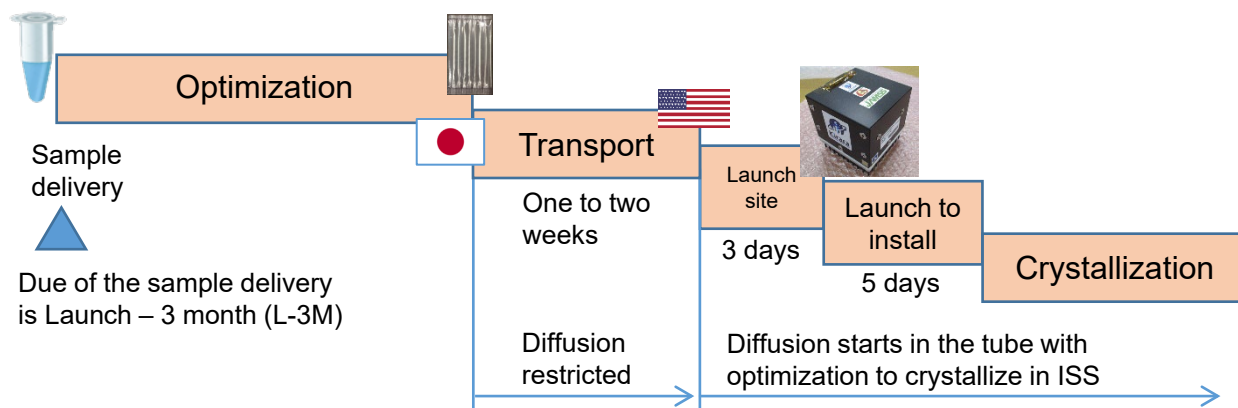
Crystallization is performed by counter-diffusion method (applying other methods more suitable for the sample if necessary) in the JAMSS Cube on the ICE Cube Facility (European Experiment Module) installed at the International Space Station (ISS).

Crystallization is performed for **about one month, set at 20degC.**

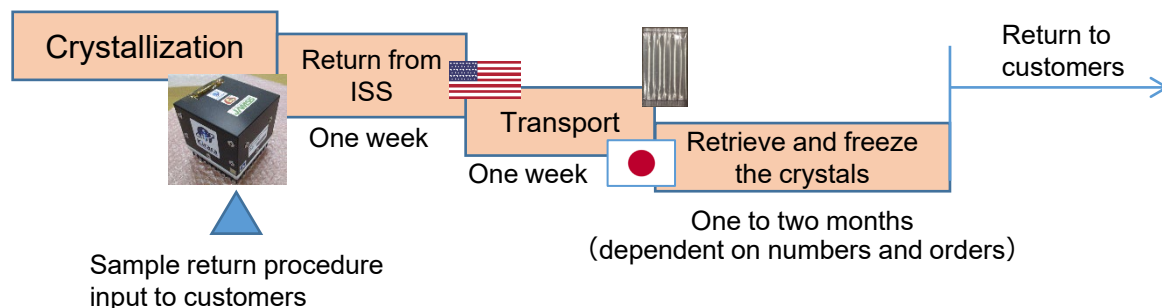
It is carried out on the ground to fill the protein solution into the capillaries, attach the gel tubes, and then fill the crystallization solution into the crystallization vessels.

After that, diffusion inside the tubes are restricted by using a stopper from the outside of the crystallization tubes. The stoppers are released in the launch site to start diffusion. With adjusting concentration of the protein solution and the crystallization solution, crystallization is expected to start in appropriate timing at the ISS.

<Timeline to crystallization in space>



<Timeline to return with frozen crystals>



*The period listed is the typical case and may change

Implementation

Crystallization Vessel

For crystallization, a container (C-SGT; right figure) consisting of two quadruple elongated cylindrical cell (crystallization tubes) made of PET sheets with high gas barrier properties is used. Up to two glass capillaries can be contained in one cell with a total length of 5.7 cm and an inner diameter of 5 mm, and crystallization conditions can be set for each bag.

For neutron analysis, capillaries made of quartz will be provided. We have several types of capillaries as shown below. We might recommend preferable one through the mission prep.

Ultimately, the best method for crystallization in space will be determined.



C-SGT

Method	CD (Counter-diffusion)				LC DM * (Large bore capillary with a dialysis membrane)				DPOC ** (Diffusion pair with osmotic concentration)
Type	0.5*35 Cap	0.7*35 Cap	2*18 LC	2*18 QLC	2*18 LC DM	2*18 QLC DM	2*32 SLC DM	2*32 QSLC DM	DPOC
	Default straight	straight	different diameter	different diameter	different diameter	different diameter	straight	straight	straight
Material	Glass	Glass	Glass	Quartz	Glass	Quartz	Glass	Quartz	silicone rubber
Osmosis membrane	—	—	—	—	+	+	+	+	—
Inner diameter (mm)	0.5	0.7	2	2	2	2	2	2	1
Length (mm)	35	35	18	18	18	18	32	32	39.5
Remark	Standard type	Larger	Large crystal	Large crystal Neutron analysis	Large crystal	Large crystal Neutron analysis	Large crystal	Large crystal Neutron analysis	Similar method with vapor diffusion Can be used for Seeding
No. of capillaries /cell	2	2	1	1	1	1	1	1	2

Implementation

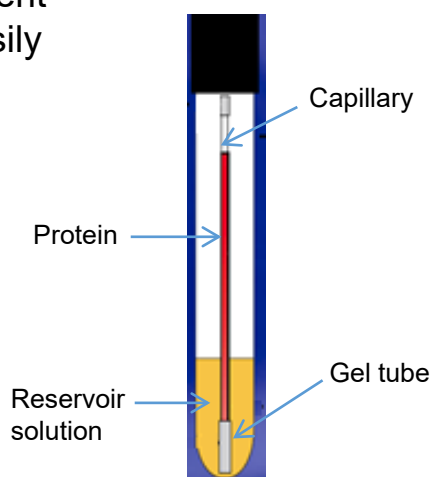
Crystallization Method

CD Method (Counter-diffusion)

The counter-diffusion method is a crystallization method in which a protein sample in a capillary and a crystallization reagent are mutually diffused. ¹⁾ Protein molecules diffuse out of the capillary and crystallization reagent molecules diffuse into the capillary to form a concentration gradient in the capillary. Within the capillary, a wide range of the pair of protein and crystallization reagent concentrations can be established over time, and crystal growth starts when the nucleation conditions is reached.

By setting the concentration of the crystallization reagent in the reservoir solution to be high, it is possible to easily study a wide range of crystal formation conditions. Therefore, one capillary is screening infinite crystallization conditions.

The counter diffusion method used in this service is the gel tube method (GT method) in which a silicon tube containing agarose gel is attached to the tip of the glass capillary. ²⁾ ,³⁾



- 1) Garcia-Ruiz, J.M., Moreno, A.: Acta Cryst., D50, 484-490(1994)
- 2) Tanaka et al., J. Synchrotron Rad., 11, 45-48(2004)
- 3) Takahashi et al., Int. J. Microgravity Sci. Appl., 36, 360107(2019)

LC DM Method (Large bore capillary with a dialysis membrane)

This technique diffuses the crystallization solution into the protein solution through the dialysis membrane between the capillary and the gel tube. Large crystals for neutron diffraction experiments can be obtained. It is also useful for reducing protein leakage from the capillary. ³⁾

DPOC Method (Diffusion pair with osmotic concentration)

It is a method of crystallization by concentration due to the difference in osmotic pressure between the solution inside the silicon tube and the crystallization solution on the outside. A silicone tube is continuously filled with a protein solution and a crystallization solution to form a diffusion pair. The method is expected to produce a change in solute concentration similar to that of the vapor diffusion method. ³⁾

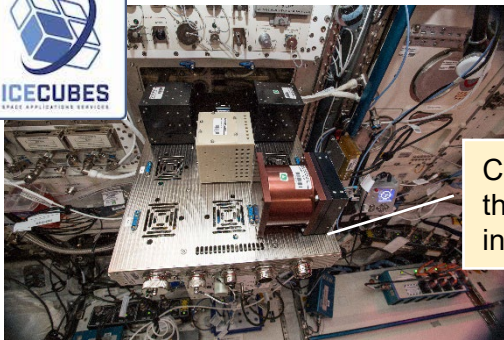
Implementation

JAMSS Cube

JAMSS Cube integrates crystallization tubes unit and incubation unit in 1U (10cm cubic). Up to 96 cylindrical cells can be mounted per unit. The temperature environment during the crystallization is 20 ± 2 degC.

When JAMSS cube arrives at the ISS, ISS crew pick up the cube from the transfer bag and install it at ICE Cubes Facility (ICF) in European module of the ISS. Then the ground team power up JAMSS Cube to start Peltier-module control to keep required temperature for crystallization. Retrieval process is almost opposite of installation.

In real time, the ground monitors current values to be input the cube to check Peltier-module working. JAMSS Cube mounts temperature loggers to confirm internal temperature after return.



ICE Cubes Facility
(ICF) in ISS

Power



Cube is located on
the tray to be pushed
inside the rack.



JAMSS
Cube

Small incubator with
Peltier-module.
Crystallization tubes
and temperature
loggers are inside.

Implementation

Deliverable

Crystals returned from space are transported to Japan. You can choose the delivery options from the following three.

The shipping location will be Confocal Science laboratory **in Japan**.

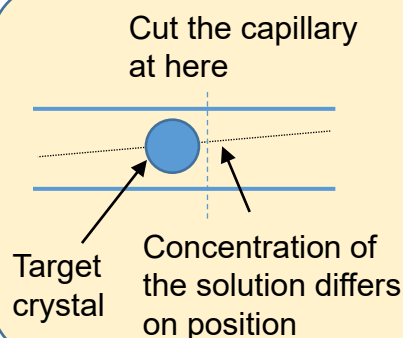
Transportation from the shipping location will be **responsible to the customers** (including customs clearance if the location is not Japan). Preferable courier company can be suggested. If X-ray diffraction is desired, it will be returned as

Options	Note
1) Crystallization tube	Some skills are required to extract crystals from the tubes. Please consult with us for prior training (an optional contract)
2) Frozen crystal As Default	It is necessary to prepare for Dry shipper (CX100, CXR500 etc.) , Dry shipper case, Shipping cane, Cryo-pin, Loop, Cryo-vial, Uni-Puck and Solutions for frozen crystals by customers. Please consult us when you use other types of equipment.
3) X-ray diffraction data	An optional contract. We uses Diamond Light Source (DLS) in the UK for X-ray diffraction. Data will be sent to the customers. If requested, structural analysis is also an option.

After returning, we will observe the grown crystals and report the condition to the customer before processing or freezing the crystal as following ratings.

1. No change
2. precipitant, amorphous, oil
3. Crystals but not good for X-ray diffraction exp.
4. Crystals good enough for X-ray diffraction exp.

We will take photos for the crystals which have rating 2 or higher of evaluation criteria above and send them to customers for their judgement and preparation of transportation.



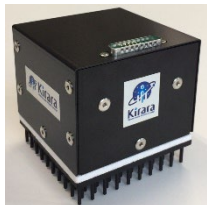
How to remove the target crystal from the capillary

Per observation, we pick up some target crystals by cutting the capillary at nearby. One important point is that we have to be careful to use the harvest solution to avoid damage on the crystal by osmotic pressure caused by the concentration difference of the solution. We calculate expected concentration by 1-D simulation for the diffusion process and adjust the reservoir solution to put the target crystal.

Implementation

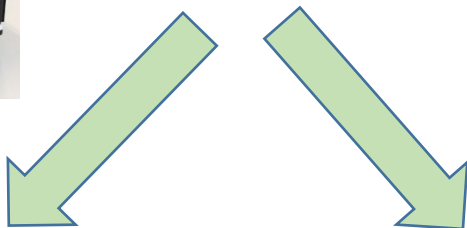
Our Team

“Kirara” service is provided with world-wide team in LEO commercialization.

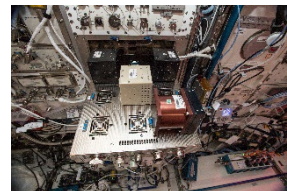


Japan Manned Space Systems Corporation
JAMSS

- ◆ Integration of “Kirara” service
- ◆ Development of JAMSS Cube (small incubator)



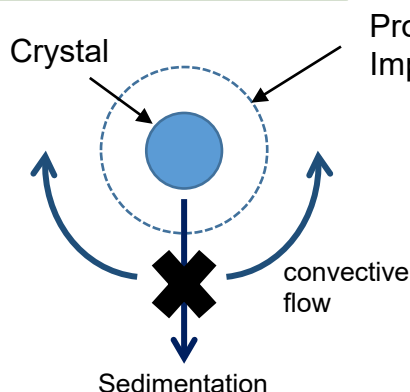
spaceapplications
SERVICES



- ◆ Long experience of protein crystallization in space since Space Shuttle era (doi:10.3390/cryst10020078)
- ◆ Manufacturing crystallization tubes
- ◆ Optimization of protein crystallization in space etc.

- ◆ ESA Commercial partner
- ◆ Services of ICE Cubes Facility for JAMSS Cube
- ◆ Marketing in Europe

Why in Space?



Protein Depletion Zone (PDZ)
Impurity Depletion Zone (IDZ)

In micro-gravity environment, crystals in the vessels do not settle down to avoid a non-uniform growth caused by the vessel wall.

It is also known that diffusion becomes superior to convection. It induces Protein Depletion Zone (PDZ) which suppresses speed of crystal growth and Impurity Depletion Zone (IDZ) which suppresses impurity uptake onto the crystal surface. PDZ and IDZ suppress dis-order in the crystal, which results in high quality crystals. ¹⁾

- ✓ No sedimentation due to gravity
- ✓ No convective flow caused by density difference

1) Y. Hashizume, et. al.; Crystals 2020, 10, 78

Precautions of Samples

Crystal Formation Temperature

Orbital crystal formation is performed at 20 ± 2 degC .

Protein Stability

Plan for about three months from sample filling to X-ray diffraction experiments. Please let us know if there is a problem with the long-term stability of the protein sample.

Mold Prevention

We recommend filter sterilization of the solution to prevent the occurrence of mold.

The addition of preservatives can affect the crystallization conditions.

In the case of use, please enter the name of preservative and the amount added on the application data sheet.

Handling of samples after the experiment

The remaining amount of the sample will be stored at our subcontractor for half a year. After half a year, we will inactivate and dispose of it properly. If you wish to return it, please fill in the remark's column of the application data sheet. Transportation from the shipping location will be **responsible to the customers** (including customs clearance if the location is not Japan).

Excerpted from Laboratory Biosafety Guidelines

Table 1. Classification of infective microorganisms by risk group

Risk Group 1 (no or low individual and community risk)

A microorganism that is unlikely to cause human or animal disease.

Risk Group 2 (moderate individual risk, low community risk)

A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited.

Risk Group 3 (high individual risk, low community risk)

A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available.

Risk Group 4 (high individual and community risk)

A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available.

Table 2. Relation of risk groups to biosafety levels, practices and equipment

RISK GROUP	BIOSAFETY LEVEL	LABORATORY TYPE	LABORATORY PRACTICES	SAFETY EQUIPMENT
1	Basic – Biosafety Level 1	Basic teaching, research	GMT	None; open bench work
2	Basic – Biosafety Level 2	Primary health services; diagnostic services, research	GMT plus protective clothing, biohazard sign	Open bench plus BSC for potential aerosols
3	Containment – Biosafety Level 3	Special diagnostic services, research	As Level 2 plus special clothing, controlled access, directional airflow	BSC and/or other primary devices for all activities
4	Maximum containment – Biosafety Level 4	Dangerous pathogen units	As Level 3 plus airlock entry, shower exit, special waste disposal	Class III BSC, or positive pressure suits in conjunction with Class II BSCs, double-ended autoclave (through the wall), filtered air

BSC, biological safety cabinet; GMT, good microbiological techniques (see Part IV of this manual)

Excerpted from Laboratory Biosafety Guidelines

Table 3. Summary of biosafety level requirements

	BIOSAFETY LEVEL			
	1	2	3	4
Isolation ^a of laboratory	No	No	Yes	Yes
Room sealable for decontamination	No	No	Yes	Yes
Ventilation:				
— inward airflow	No	Desirable	Yes	Yes
— controlled ventilating system	No	Desirable	Yes	Yes
— HEPA-filtered air exhaust	No	No	Yes/No ^b	Yes
Double-door entry	No	No	Yes	Yes
Airlock	No	No	No	Yes
Airlock with shower	No	No	No	Yes
Anteroom	No	No	Yes	—
Anteroom with shower	No	No	Yes/No ^c	No
Effluent treatment	No	No	Yes/No ^c	Yes
Autoclave:				
— on site	No	Desirable	Yes	Yes
— in laboratory room	No	No	Desirable	Yes
— double-ended	No	No	Desirable	Yes
Biological safety cabinets	No	Desirable	Yes	Yes
Personnel safety monitoring capability ^d	No	No	Desirable	Yes

^a Environmental and functional isolation from general traffic.

^b Dependent on location of exhaust (see Chapter 4).

^c Dependent on agent(s) used in the laboratory.

^d For example, window, closed-circuit television, two-way communication.

Conditions of the Services

For entry

Followings must be satisfied for service entry

- ◆ Crystals are already obtained in some method. If not yet, we can introduce another service to support crystallization before entering Kirara.
- ◆ Target protein, its raw materials and the manufacturing process are assured to be safe. No toxic and pathogenic samples.
- ◆ Not subject to import / export restrictions of U.S. as well as Japan.

Cross-waiver of liability

In the experiments and services that use the International Space Station, the contents of the international treaty * concerning the mutual abandonment of the right to claim damages by participating countries and related parties are applied. In the case of this service, the right to claim is also abandoned between the parties concerned.

* Intergovernmental Agreement (IGA) on Space Station Cooperation, along with representatives from Russia, Japan, Canada and participating countries of the European Space Agency

Intellectual property

All deliverables and intellectual property rights obtained from this service belong to the customers. JAMSS and subcontractors will not claim any right or interest in the results.

Change and Termination of the services

Due to delays in the launch and return of spacecraft, space experiments may be canceled or rescheduled.

In case critical issues are found to continue our services before launch as following but not limited to, we may terminate services for the dedicated samples with consultation to the customers.

- (1) We cannot verify crystallization of the samples delivered in a certain period.
- (2) Space mission is not valid for the samples to be crystalized.
- (3) We cannot get agreement from the customers to disclose additional information of the samples per request from NASA/ESA.
- (4) Samples are prohibited items in US customs law.

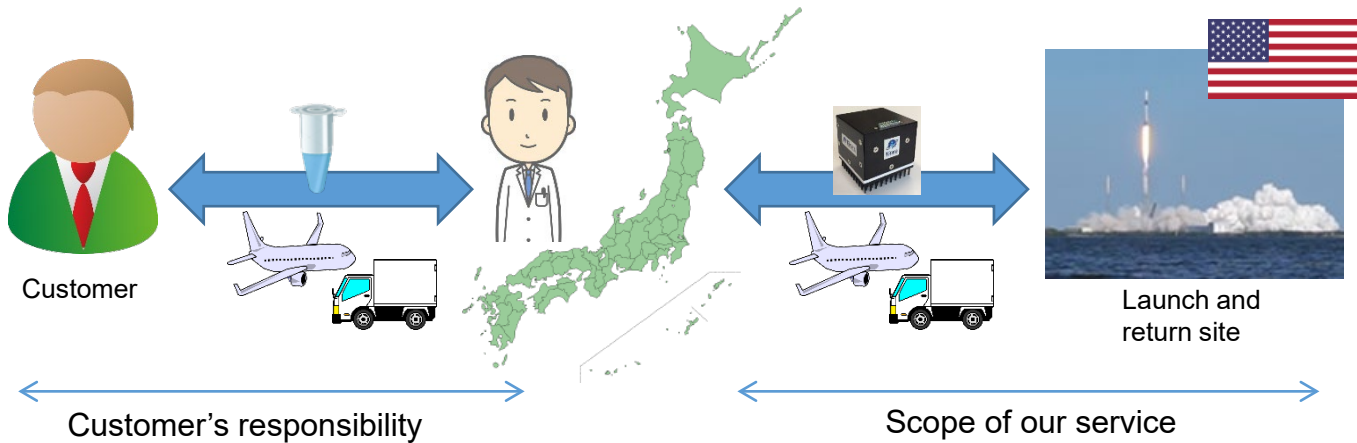
Conditions of the Services

Roles and Responsibilities

Title	Contents	Customer	JAMSS
Application	• Fill in the order form of “Kirara” and send it to JAMSS (via Space Apps). • It includes information of the samples for safety review of International Space Station. If required by NASA/ESA, we may ask more info to be provided.	X	-
Samples prep and delivery	• Prepare for proteins with sufficient purity and concentration for crystallization. • Prepare for crystallization solutions with appropriate compositions for crystallization. • Deliver the proteins and crystallization solutions to the site designated by JAMSS. • Send data to optimize the crystallization conditions on-orbit etc. * Customers are responsible for shipping costs.	X*	-
Documentation for safety review, transport and ops	• Prepare and apply for documents related to the safety review of the samples. • Prepare and apply for documents related to transportation, including import and export of the samples. • Prepare and apply for documents related to the onboard operations.	-	X
Optimization (before launch)	• Confirm crystallization using methods provided by customers. • Optimize conditions suitable for on-orbit crystallization using the gel tube method. * We may contact you in the process of examining crystallization conditions.	-*	X
Preparation for SGT	• Fill the protein solution into the capillaries, attach the gel tubes, and the fill the crystallization solutions into the crystallization vessels (SGT).	-	X
Transport to the launch site	• Transport samples in SGT to the launch site with conditioned temperature.	-	X
Crystallization in ISS	• Coordinate launch of JAMSS Cube with the samples to ISS. • Coordinate missions of protein crystallization on-orbit including return.	-	X
Transport from NASA	• Transport deliverables from NASA facility to Japan with conditioned temperature.	-	X
Result of crystallization	• Check the crystal growth status and report to the customer	-	X
Retrieve and freeze crystals	• The conditions for taking out the obtained crystal are determined, and the crystals are retrieved • Freeze the crystals for X-ray diffraction * The customer needs to prepare the necessary equipment and solutions with cryoprotectant for freezing and transporting them.	-*	X
X-ray diffraction Structure analysis (Optional)	• Diffraction data is acquired by DLS, UK using frozen crystals. • Perform structure analysis from diffraction data if requested.	-	Option
Transport to the customer's site	• Deliver deliverables to customers from our designated facilities. * Customers are responsible for shipping contracts and costs.	X*	-

Reference Information

Transportation



It is customer's responsibility to prepare for transportation between your site and JAMSS designated location in Japan. It includes preparation of all documents for shipping and payment of taxes not only when sending your cargo but when return.

Custom tax is exempted according to types of the contents but consumption tax is applied for goods higher than 10,000JPY in custom clearance of Japan. If it is temporal export from your site and no change is added when return, the tax you pay can be refunded per the following process:

https://www.customs.go.jp/english/c-answer_e/imtsukan/1606_e.htm

We can help preparing for the application documents but make sure that your shipping company and their service support the process. **Some major shipping company's service NEVER support the refunding process in Japan.**

Reference info:

Prohibited items to import to and export from Japan

https://www.customs.go.jp/english/c-answer_e/kinseihin/2001_e.htm

https://www.customs.go.jp/english/c-answer_e/kinseihin/2501_e.htm

Japan CSCL(Chemical Substances Control Law) and the database

https://www.meti.go.jp/policy/chemical_management/english/cscl/index.html

https://www.nite.go.jp/chem/jcheck/top.action?request_locale=en

Information on Cartagena Protocol domestic (Japanese) law for LMO

http://www.biodic.go.jp/bch/english/e_index.html