Application data sheet #09

Constant temperature incubator SWING INUBATOR SW-060

An example of ordinary temperature defrosting and proposing to improve the reproducibility in room temperature reactions

Collect basic data for using this product by defrosting reagents and samples under ordinary (room) temperature

Overview

When experiments are conducted in life science, a regular task involves defrosting frozen reagents and samples. For example, quick defrosting by soaking them in a water bath at +37℃ and naturally defrosting them in ice, depending on the reagents involved. If heating or soaking is not desirable, they can be defrosted naturally on a desk (at room temperature.) In either way, the time necessary for defrosting is not strictly determined, as it is not necessary in the first place or because the time necessary for defrosting is not constant because of a fluctuating room temperature. However, for tasks where the promotion of an efficient process is necessary such as the entrusted service of gene analysis, there are requirements to determine the time required for a defrosting operation. In this case, a water bath is not favored as it requires additional effort such as refilling water and the wiping of wet reagent/sample cases. In addition, a water bath is not really suitable for the defrosting of reagents and samples that do not suit humidification because of equipment characteristics. Also, as described above, even if room temperature changes when air conditioned, depending on day and night or seasons, with defrosting at room temperature, it is possible that the time necessary cannot be reproduced.

The constant temperature incubator SWING INCUBATOR SW-060 featured in this document can be a solution to this. Therefore, we use this Product to collect basic data that can be seen as a reference when reagents and samples are defrosted at a temperature that is the same as the room temperature. What the room temperature actually means is the degree of centigrade described below.

About the definition of room temperature and the decision for precise defrosting temperature

We use the term room temperature but with regard to the storage temperature of food stuff and pharmaceuticals, the phrase ordinary temperature is used. What temperatures are room temperature and ordinary temperature? Below are the definitions and custom uses:

Source/Field	Japanese Pharmacopoeia [1] (Temperature to be used for tests and storage)	Japan Industrial Standards (JIS)[2] (Standard atmospheric conditions for testing)	Microbiology (custom usage)	Life science in general[3]
Room temperature	+1℃～+30℃	—	+25℃	+20℃
Ordinary temperature	+15℃～+25℃	20℃±15℃ (+5℃～+35℃)	+25℃	+20℃
Temperature at standard atmospheric conditions	+20℃	+20℃ , +23℃ , +25℃ depending on test purpose	—	—

According to Japanese Pharmacopoeia, room temperature has a wide range of temperatures, while ordinary temperature is specified in a narrower range than room temperature. At the same time, room temperature is not defined in the specification of a testing location in JIS, while ordinary temperature is a range of temperatures close to the room temperature as per Japanese Pharmacopoeia. For a "temperature in a standard condition," three definite temperatures are given. Generally, when looking at customs in microbiology and other life sciences, both room temperature and ordinary temperature are recognized as +25℃ or +20℃ . Taking all of these things into consideration, we can understand that a range of temperature inspired from the term room temperature is close to the range of ordinary temperature in Japanese Pharmacopoeia. The protocol of "reaction at room temperature" is not likely to assume +1℃ . We therefore use ordinary temperature to describe so-called room temperature herein, while the defrosting temperature at the ordinary temperature is set to +25℃ to conduct defrosting tests.

Test method

■Investigation of the time necessary for ordinary temperature defrosting of ice and the confirmation of reproducibility

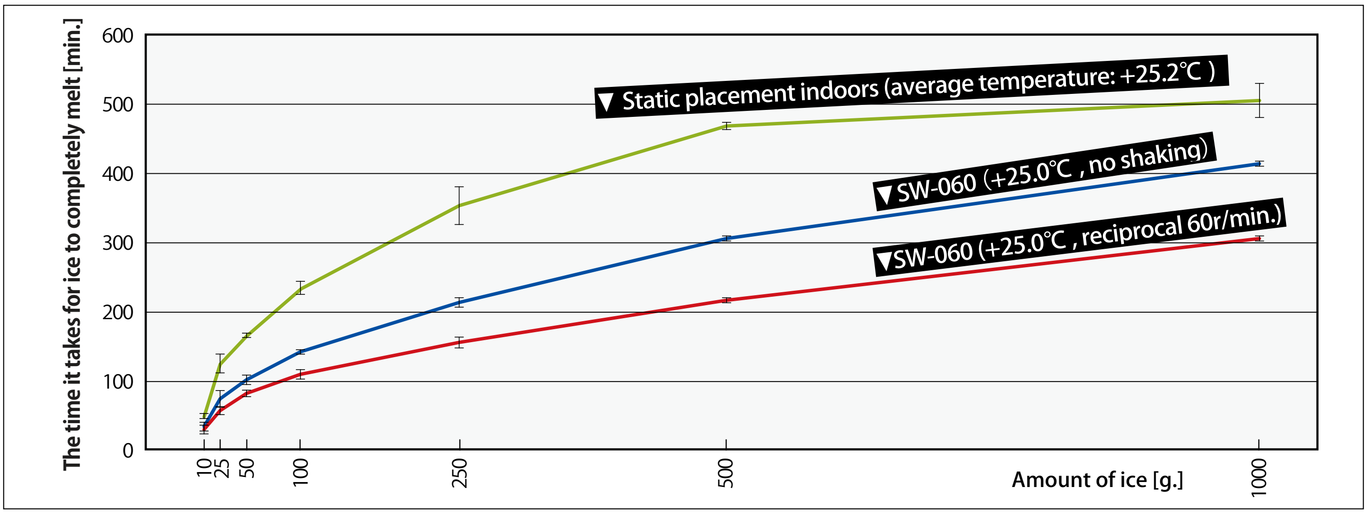
As shown in the table on the right, we prepared containers, and then put tap water into them and froze them ("ice"). We visually checked the time for the ice to fully melt at five-minute intervals in three different conditions: (1) setting the container in the SW-060 at +25℃ and shaking it, (2) the same but no shaking, and (3) statically placing the container in an air-conditioned room*. It is possible to calculate a theoretical value of time for the ice to fully melt, and we thought that a comparison with actual values may be useful for consideration. However, getting the parameters necessary for a calculation is rather difficult, so we used actually measured values only. Should we have an opportunity, we will make an update to this document.

*Room temperature during the test period was +24.3 ℃ at the lowest, +26.2 ℃ at the highest, and +25.2℃ on average.

10g	10g of tap water in 10mL disposable centrifuge tube	Frozen for a night or longer at -20℃
25g	25g of tap water in 50mL disposable centrifuge tube	
50g	50g of tap water in a 100mL PE bottle	
100g	100g of tap water in a 250mL PE bottle	
250g	250g of tap water in a 250mL PE bottle	
500g	500g of tap water in a 1,000mL PE bottle	
1000g	1,000g of tap water in a 1,000mL PE bottle	

●When water is frozen, its volume increases by one-eleventh, therefore the volume of water and ice are represented in mass [g].

Results and discussion



We defined the ordinary temperature defrosting as thawing at +25 ℃ , and each amount of ice was defrosted. The melting speed was fastest when shaking with the SW-060, followed by no shaking in the SW-060, and static placement indoors. The inside air of the SW-060 was forced to circulate to improve the temperature distribution. This promotes heat exchange around the ice container, so the melting speed was considered to become faster than static placement indoors. The effect of shaking is similarly regarded as the promotion of heat exchange. Next is the reproducibility of the defrosting time. As for static placement indoors, errors were comparatively large, and the linearity in the relationship between the amount of ice and the defrosting time was lower compared to cases where containers were put into the SW-060. This was likely because there was rarely any influence from the fluctuations of the indoor temperature or any factor to promote heat exchanges near the container (Indoor temperatures during the test period was +24.3℃ at the lowest, +26.2℃ at the highest, and +25.2℃ on average). On the other hand, for the three conditions, there were no special linearity with the defrosting time when the ice was 10 to 50 g. This could be related to the material and thickness of the container. Given below are the specifications of the containers used. When looking at a mathematical expression to calculate the theoretical defrosting time of ice (omitted here), the thermal conductivity and thickness of the container are related. Polystyrene and polypropylene have no big difference in terms of thermal conductivity, but high-density polyethylene has a thermal conductivity that is a few times better. When a container becomes bigger, it will become thicker to increase the resistance of heat transfer. We used high-density polyethylene containers for 100 mL or above. In addition, the bigger the container becomes, the wider the heat transfer area will be. It is therefore considered that thanks to the interaction of these parameters, the above graph was obtained.

As an air container, the SW-060 takes more time to defrost than a water container, but as described above, it has unique advantages and needs as an air container. In this test, we were able to confirm that the SW-060 has advantages in terms of reproducibility and heat exchange compared to static placement indoors. We think that it can be utilized for ordinary temperature defrosting and such protocols as "reactions at room temperature."

*0.17 to 0.19 in some literature

Amount of ice	Container used	Specifications of container used
10g	10 mL disposable centrifuge tube	Polystyrene (Thermal conductivity: 0.10 to 0.14[W/(m·K)]), thickness: about 1.0 mm
25g	50 mL disposable centrifuge tube	Polypropylene (Thermal conductivity: 0.12*[W/(m·K)]), thickness: about 1.0 mm
50g	100 mL PE bottle	High-density polyethylene (Thermal conductivity: 0.46 to 0.52[W/(m·K)]), Thickness: about 1.5 mm
100g	250 mL PE bottle	High-density polyethylene (Thermal conductivity: 0.46 to 0.52[W/(m·K)]), Thickness: about 1.5 mm
250g	250 mL PE bottle	High-density polyethylene (Thermal conductivity: 0.46 to 0.52[W/(m·K)]), Thickness: about 1.5 mm
500g	1,000 mL PE bottle	High-density polyethylene (Thermal conductivity: 0.46 to 0.52[W/(m·K)]), Thickness: about 2.0 mm
1000g	1,000 mL PE bottle	High-density polyethylene (Thermal conductivity: 0.46 to 0.52[W/(m·K)]), Thickness: about 2.0 mm

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Constant temperature incubator
Swing incubator SW-060

Temperatures near room temperature such as +25 ℃ are adjusted with high precision. Freon-free electronic heating/cooling system. Shaking Kits are useful for defrosting, and reactions at room temperature and hybridization up to +60℃ are also available.

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References

1) The Japanese Pharmacopoeia, 17th edition (March 7, 2016, GENERAL NOTICES No. 16)
2) JIS Z 8703
3) Nakayama, Hiroki & Takahito Nishikata, Bio Experiment Illustrated, 1, 90

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