

# Example ① Freeze crushing of various samples including inanimate samples

**Embrittlement by freezing enables strong crushing.**

**The freeze crushing with  $\mu$ T-48 is also suitable for Obligatory anaerobe samples.**



•Freeze Crusher  $\mu$ T-48 --> P.105

## Test results

- Freezing method Immerse the vessels with the sample and crusher into liquid nitrogen (2.0 mL: Vessel holder) and then freeze them.
- Rushing time 30 sec (Additional 30 sec if not completely crushed)
- Judgment whether sample is crushed Whether powder forms or nearly forms (Cut samples into any size that can be put in vessels).
- Vessels
  - Safe-Lock tube 2.0 mL ...Marketed product (Made by Eppendorf)
  - Metal crusher.....Included in Optional 48 pcs-holder for  $\mu$ T-48 (used in this experiment)
  - Stainless steel-made strong crush vessels .....Optional parts (Dedicated crusher is included.)

### Chicken thigh



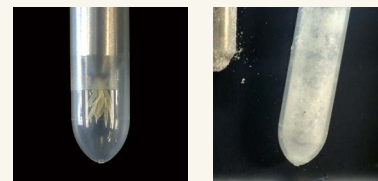
Vessels: Safe-Lock tube 2.0 mL  
Sample volume: 0.1 g  
Shaking speed: 1200 r/min  
Crushed with: Metal crusher

### Human hair



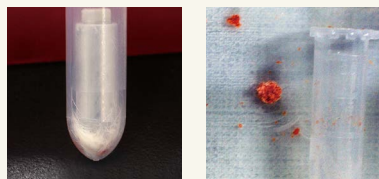
Vessels: Safe-Lock tube 2.0 mL  
Sample volume: 0.1 g  
Shaking speed: 1200 r/min  
Crushed with: Metal crusher

### Human nails



Vessels: Safe-Lock tube 2.0 mL  
Sample volume: 0.2 g  
Shaking speed: 1200 r/min  
Crushed with: Metal crusher

### Mouse skin (with body hair)



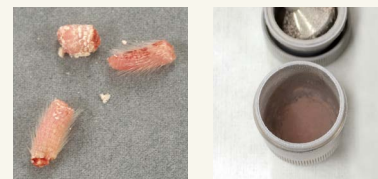
Vessels: Safe-Lock tube 2.0 mL  
Sample volume: 0.2 g  
Shaking speed: 1200 r/min  
Crushed with: Metal crusher

### Mouse heart



Vessels: Safe-Lock tube 2.0 mL  
Sample volume: 0.2 g  
Shaking speed: 1200 r/min  
Crushed with: Metal crusher

### Mouse tail



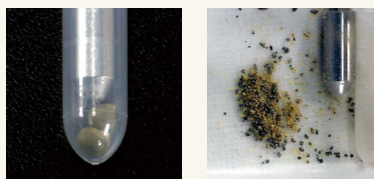
Vessels: Stainless steel-made strong crush vessel  
Sample volume: 1 g  
Shaking speed: 1000 r/min  
Crushed with: Dedicated crusher

### Hypocotyl of Radish



Vessels: Safe-Lock tube 2.0 mL  
Sample volume: 0.2 g  
Shaking speed: 1200 r/min  
Crushed with: Metal crusher

### Okra seeds



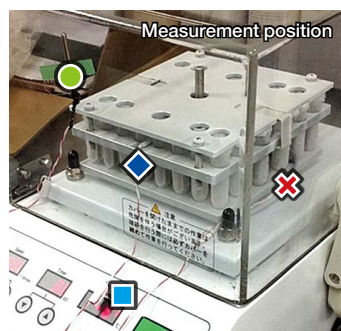
Vessels: Safe-Lock tube 2.0 mL  
Sample volume: 2 pcs  
Shaking speed: 1200 r/min  
Crushed with: Metal crusher

### Hard rubber (Polychloroprene)

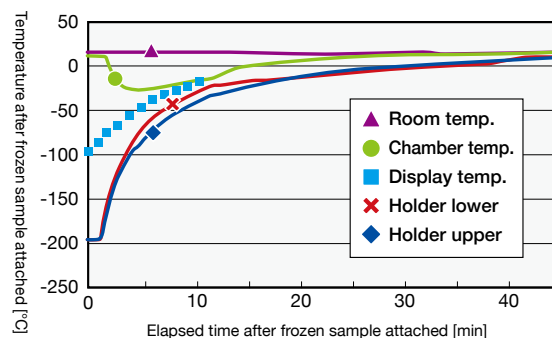


Vessels: Stainless steel-made strong crush vessel  
Sample volume: 2 g  
Shaking speed: 1000 r/min  
Crushed with: Dedicated crusher

## Frozen sample/Holder temperature (Reference)



The 48 pcs-holder for 2 mL tube TH-0248T with 48 tubes and Metal crusher that was frozen with liquid nitrogen was attached to the unit. Then, the temperature change of each part while shaking at 1200 r/min was measured. The cryogenic temp. was completely kept for 30 to 60 sec which was required for crushing. The display temp. indicates the temp. stage of the top surface on which the holder is placed.



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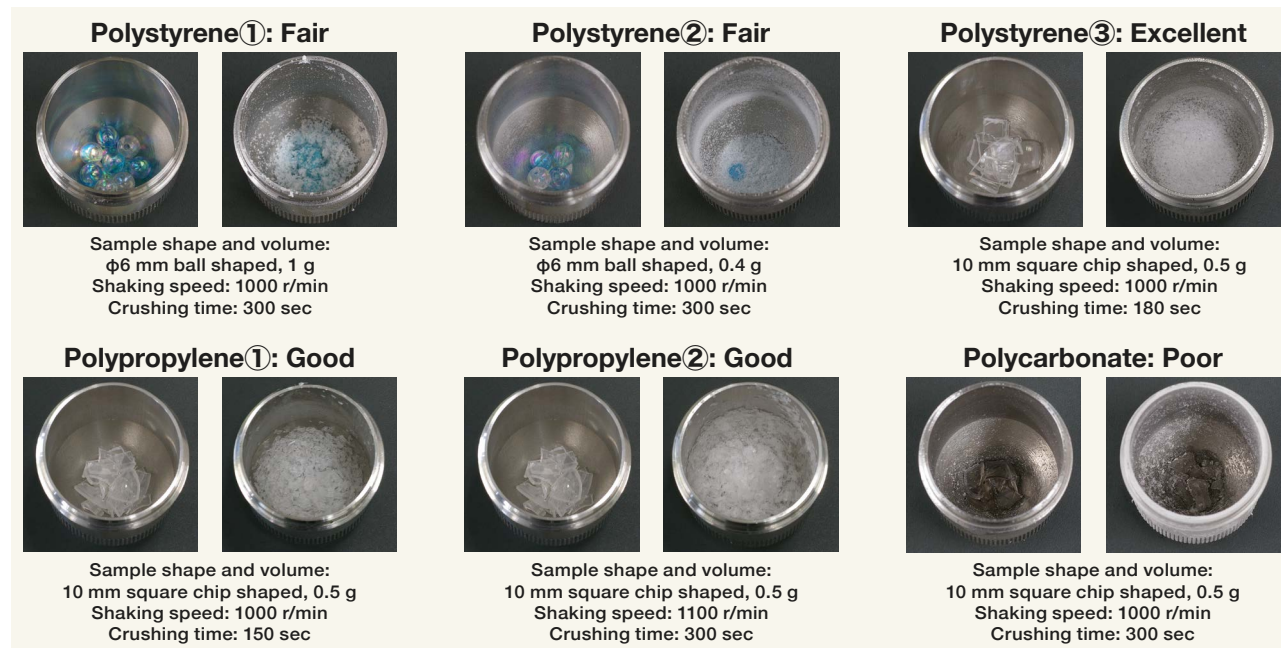
**Freeze crushing of Plastic samples using Freeze crusher  $\mu$ T-48 with Stainless steel-made strong crush vessel.**

• Freeze Crusher  $\mu$ T-48 --> P.105



### Results and Examination

We tried some crushing of samples such as polystyrene, polypropylene, and polycarbonate that are well known. Each result is as follows.



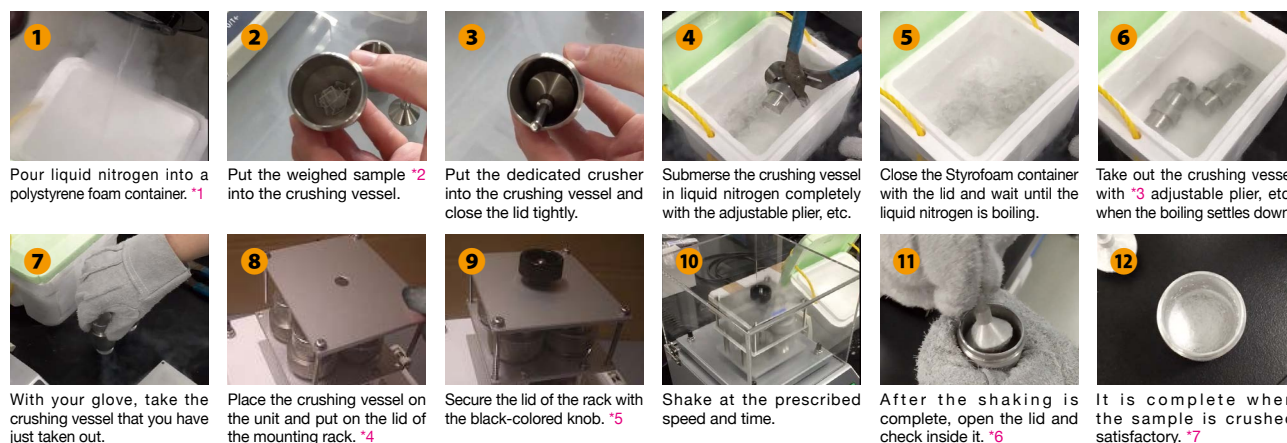
Polystyrene was able to be powdered completely (③). However, in case of the φ6 mm ball-shaped sample, there were large fragments that remained at a certain rate even after trying with different amounts and crushing times (①②). It seems that the ball-shaped sample remained uncrushed if it is stuck in upward of the crusher.

The result suggests that the shape of the sample is better to be like a chip shape (or a tablet shape) Polypropylene in order to be crushed into fine fragments. However, it was not crushed into powder (①). In order to improve (although the shaking speed limit is exceeded when using a strong crushing container), the shaking speed was performed at 1100 r/min for 5 minutes. It becomes fine, but like a braided piece of cotton (②). At this stage, it becomes difficult to collect unless suspended in a solvent.

Polycarbonate proved to be difficult to crush. Even if the shaking speed was reduced to 1100 r/min or by reducing sample amount, the result of this experiment was that only a small amount of powder was produced and the chip shape remained almost unchanged.

### Freeze crushing procedure when using stainless steel-made strong crushing vessel

An adjustable plier is useful for taking the Stainless steel-made strong crushing vessel (referred to as crushing vessel) in and out of the liquid nitrogen. Make sure to wear leather gloves when touching the frozen crushing vessel or the vessel holder that has become cold by contacting the frozen vessel. Make sure to ventilate the room well when using liquid nitrogen as there is risk to get Anoxia unknowingly because vaporized liquid nitrogen can become a huge volume of nitrogen gas.



\*1. Desirable to use the minimum-sized polystyrene foam container that the required number crushing vessels can be immersed to minimize the amount of liquid nitrogen used.

\*2. The processing capacity of the crushing vessel is 1.2 g per 1 pc while it is better to make it to 0.5 g per 1 pc for plastic samples (Up to 1 g polystyrene can easily be crushed by freezing).

\*3. Wait for at least 2 minutes after the boiling is settled out to freeze the sample in the crushing vessel sufficiently.

\*4. Place at least two "frozen" Stainless steel-made strong crushing vessels for balance and secure fixation. Because metal shrinks when frozen, both vessels must be frozen to ensure a firm fixation.

\*5. In December 2017, the rack was changed to a new type that does not require thumbscrews to secure vessels.

\*6. The crushed sample may stick to the crusher so tap it with the inner wall of the vessel to drop it.

\*7. If the crushing is insufficient, return the crusher to the unit to freeze it and shake again.