

JAXA Biospecimen List 1 (MHU-1)

Please enter both “Tissue No.” and “Tissue name” on your “Biospecimen Request Form.”

Up to three kinds of tissues can be requested per this Material Transfer Agreement (MTA).

Tissue No.	Tissue name	Sample No.	Gravity condition	Treatment	Storage
1-4	Skin A	1-6 7-12 13-18	A1G (1-6) Micro G (7-12) 1G (13-18)	PFA fixation ↓ MeOH exchange	-30°C 15ml tube x 18
1-5	Skin B	1-6 7-12 13-18	A1G (1-6) Micro G (7-12) 1G (13-18)	PFA fixation ↓ MeOH exchange	-30°C 15ml tube x 18
1-11	Tail A (Skin)	1-6 7-12 13-18	A1G (1-6) Micro G (7-12) 1G (13-18)	PFA fixation ↓ MeOH exchange	-30°C 15ml tube x 18
1-16	Auricles	1-6 7-12 13-18	A1G (1-6) Micro G (7-12) 1G (13-18)	PFA fixation ↓ MeOH exchange	-30°C 15ml tube x 12 1.5ml tube x 6

- **Gravity Condition**

A1G: Artificial 1 G on ISS (September 2016)

Micro G: Micro Gravity on ISS (September 2016)

1G: Ground Control (October 2016)

- **Treatment Method**

PFA fixation→MeOH exchange:

1. After dissection, tissues were fixed in 4% paraformaldehyde (PFA, WAKO) in the US in September 2016. After 4% PFA fixation (1 day at 4°C), tissues were washed with phosphate buffered saline (PBS, GIBCO) and stored at 4°C.
2. Tissues were transported to the JAXA's laboratory in Japan.
3. Three weeks after PBS exchange, tissues were fixed again in 4% PFA at 4°C for 2 days, and washed with PBS. Tissues were stored at 4°C.
4. Two weeks after washed with PBS, MeOH exchange was gradually performed from 25% to 100% methanol (PBS→25% MeOH→50% MeOH→75% MeOH→100% MeOH) in October 2016, and tissues are stored at -30°C.

- **Reference**

Development of new experimental platform 'MARS'—Multiple Artificial-gravity Research System

—to elucidate the impacts of micro/partial gravity on mice

Sci Rep. 2017 Sep 7;7(1):10837. doi: 10.1038/s41598-017-10998-4. (Shiba D et al., 2017)

JAXA Biospecimen List 2 (MHU-2)

Please enter both “Tissue No.” and “Tissue name” on your “Biospecimen Request Form.”

Up to three kinds of tissues can be requested per this Material Transfer Agreement (MTA).

Tissue No.	Tissue name	Sample No.	Gravity condition	Treatment	Storage
2-6	Femur skin D	Micro G, 1-5 A1G, 1-6 GC 1-6	Micro G A1G 1G	PFA fixation ↓ MeOH exchange	-30°C 15ml tube x 17
2-14	Auricles	Micro G, 1-5 A1G, 1-6 GC 1-6	Micro G A1G 1G	PFA fixation ↓ MeOH exchange	-30°C 15ml tube x 18

● Gravity Condition

A1G: Artificial 1 G on ISS (September 2017)

Micro G: Micro Gravity on ISS (September 2017)

1G: Ground Control (March 2018)

● Treatment Method

PFA fixation→MeOH exchange:

1. After dissection, tissues were fixed in 4% paraformaldehyde (PFA, WAKO) in the US in September 2017.
2. After 4% PFA fixation (1 day at 4°C), tissues were washed with phosphate buffered saline (PBS, GIBCO).
3. Tissues were transported to the JAXA's laboratory in Japan.
4. 40 days after washed with PBS, MeOH exchange was gradually performed from 25% to 100% methanol (PBS→25% MeOH→50% MeOH→75% MeOH→100% MeOH) in October 2017, and tissues are stored at -30°C.

● Reference

Dietary intervention of mice using an improved Multiple Artificial-gravity Research System (MARS) under artificial 1g

NPJ Microgravity. 2019 Jul 8;5:16. doi: 10.1038/s41526-019-1077-0. (Matsuda C et al., 2019)

JAXA Biospecimen List 3 (MHU-3)

Please enter both "Tissue No." and "Tissue name" on your "Biospecimen Request Form."

Up to three kinds of tissues can be requested per this Material Transfer Agreement (MTA).

Tissue No.	Tissue name	Sample No.	Gravity condition	Treatment	Storage
3-3	Dorsal Skin (Upper) B (Right)	1-12	Micro G (1-12)	LN2	-80°C 5ml tube x 24
		GC 1-12	1G (GC 1-12)		
3-4	Dorsal Skin (Upper) B (Left)	1-12	Micro G (1-12)	LN2	-80°C 5ml tube x 24
		GC 1-12	1G (GC 1-12)		
3-15	Auricles (Right)	1-12	Micro G (1-12)	LN2	-80°C 5ml tube x 24
		GC 1-12	1G (GC 1-12)		
3-16	Auricles (Left)	1-12	Micro G (1-12)	LN2	-80°C 5ml tube x 24
		GC 1-12	1G (GC 1-12)		
3-19	Seminal gland (Right)	1-12	Micro G (1-12)	LN2	-80°C 2ml tube x 24
		GC 1-12	1G (GC 1-12)		
3-20	Seminal gland (Left)	1-12	Micro G (1-12)	LN2	-80°C 2ml tube x 24
		GC 1-12	1G (GC 1-12)		
3-23	Blood clot	1-12	Micro G (1-12)	LN2	-80°C 1.5ml tube x 24
		GC 1-12	1G (GC 1-12)		

- **Gravity Condition**

Micro G: Micro Gravity on ISS (May 2018)

1G: Ground Control (October 2018)

- **Treatment Method**

LN2: After dissection, tissues were frozen in Liquid Nitrogen in May 2018.

- **Genotype**

The MHU-3 experiments were conducted using two types of mice: Wild type (WT) and Nrf2 Knock Out (Nrf2) mice. Refer to the table below for consistency between the sample No. and genotype.

Micro G No.	1	2	3	4	5	6	7	8	9	10	11	12
Genotype	Nrf2	Nrf2	WT	Nrf2	WT	WT	WT	WT	Nrf2	WT	Nrf2	Nrf2

1G (GC) No.	1	2	3	4	5	6	7	8	9	10	11	12
Genotype	Nrf2	Nrf2	WT	Nrf2	WT	WT	WT	WT	Nrf2	WT	Nrf2	Nrf2

- **Reference**

Space Travel of Knockout Mice Demonstrates Contribution of Nrf2 to Maintenance of Homeostasis

Communications Biology. 2020 Sep 8;3(1):496. doi: 10.1038/s42003-020-01227-2. (Yamamoto M et al. 2020)