# JAXA Biospecimens Sharing List 1 (MHU-1)

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

The number of maximum requests is 3 tissues from all MHU missions.

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

#### [Abbreviations]

A1G: Artificial 1 G on ISS PFA fixation; 4% paraformaldehyde (WAKO) fixation after dissection in USA (2016.9)

Micro G: Micro Gravity on ISS MeOH exchange: Transport to JAXA and exchange for 100% methanol (2016.10)

1G: Ground Control

#### [Method: Fixation and exchange]

**PFA fixation**: After 4% PFA fixation (1 day at 4°C), exchange for phosphate buffered salts (PBS) and transport to JAXA (storage at 4°C). Three weeks later from PBS exchange, second fixation by 4% PFA (2 days at 4°C), and PBS re-exchange (storage at 4°C).

**MeOH exchange**: Two weeks later from PBS re-exchange, exchange for MeOH (PBS $\Rightarrow$ 25% MeOH $\Rightarrow$ 50% MeOH $\Rightarrow$ 75% MeOH  $\Rightarrow$ 100% MeOH),and storage 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 sample share List 2 (MHU-2)

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

#### The number of maximum requests is 3 tissues from all MHU missions.

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

#### [Abbreviations]

A1G: Artificial 1 G on ISS PFA fixation: 4% paraformaldehyde (WAKO) fixation after dissection in USA (2017.9)

Micro G: Micro Gravity on ISS MeOH exchange: Transport to JAXA and exchange for 100% methanol (2017.10)

1G: Ground Control (2018.3)

#### [Method: Fixation and exchange]

PFA fixation: After 4% PFA fixation (1 day at 4°C), exchange for phosphate buffered salts (PBS) and transport to JAXA

**MeOH exchange**: Forty days later from PBS exchange, exchange for MeOH (PBS⇒25% MeOH⇒50% MeOH⇒75% MeOH⇒

100% MeOH), and storage at -30°C

#### Reference

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

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

# JAXA sample share List 3 (MHU-3)

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

The number of Maximum requests is 3 tissues from all MHU missions.

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

### [Abbreviations]

Micro G: Micro Gravity on ISS 1G: Ground Control (at JAXA, 2018.10) LN2: Liquid Nitrogen freezing after dissection

## [Genotype]

In MHU3, we reared 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
GC No.	GC 1	GC 2	GC 3	GC 4	GC 5	GC 6	GC 7	GC 8	GC 9	GC 10	GC 11	GC 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)