Application data sheet #05

CO_2 / Multi-gas incubator *Prescyto* MG-71C/M

An example involving the examination of antibacterial material and low-oxygen culture in cells

In particular, for people starting cell culture and low-oxygen culture: performance examination data of this Product

About the Prescyto MG-71 series

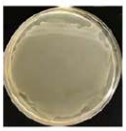
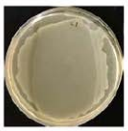
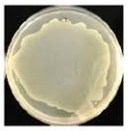
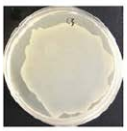
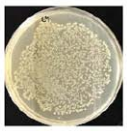
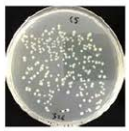
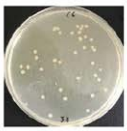
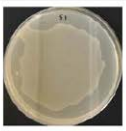
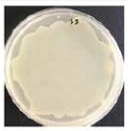
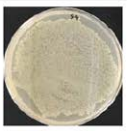
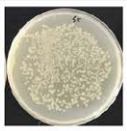
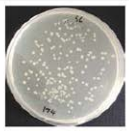
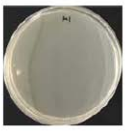
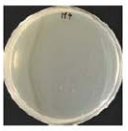
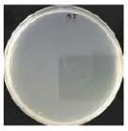
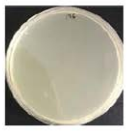
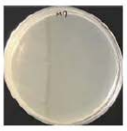
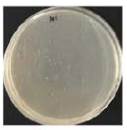
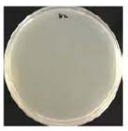
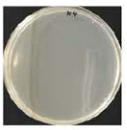
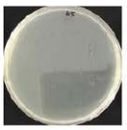
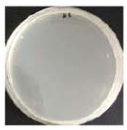
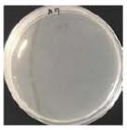
Both the Prescyto MG-71 CO2 incubator and the Prescyto MG-71M multi-gas incubator have been launched as modified models of MG-70C and MG-70M, respectively. The incorporated gas changer is omitted, but instead, improvements such as the reduction of gas consumption, strengthening the protection of gas concentration control sensors, and also the antibacterial coating of the shelf plate has been added. With the MG-71M, which is a multi-gas type, functions convenient for a low-oxygen culture are enhanced, i.e. Low-Oxygen Booster. To turn the inside into a low-oxygen state, nitrogen gas is fed to expel any air containing oxygen, and by reviewing the introduction path of the nitrogen gas, a low-oxygen state can now be achieved four times faster than the older model (about 15 minutes from atmospheric concentration to 1% O2). Assisted by the fact that this Product is a small model with an inside volume of 53 L, it is faster than other manufacturers' multi-gas incubators. Naturally, a resumption of oxygen concentration after changes from the opening or closing of the door is done quickly. We firmly believe that using the MG-71M will beneficial when a precision low-oxygen culture is required.

Examination of antibacterial coating

〈The shelf plate of both MG-71C and MG-71M have antibacterial coating. The effect of this antibacterial coating was verified by a method specified in JIS 2801 Antibacterial Products-Test for antibacterial activity and efficacy (partially modified in consideration of the convenience of experiment.)

1. Base materials sterilized by ultraviolet radiation (shelf plate with antibacterial coating * that is the subject of verification, normal shelf plate for comparison, and a bacteria culture dish as a negative reference) were prepared.
2. 100 μL of liquid containing coliform bacteria (7 x 104 cells/mL) was dropped on each base material, which was then covered with a lid of a 1.5 mL micro tube for 24 hours of a culture inside an MG-71C at +37° C.
3. 50 μL of cultivated fungus liquid was collected to create a dilution series from 10-1 to 10-7. 100 μL was applied to each LB agar medium and cultivated overnight at +37℃ .
4. Number of colonies grown were counted.

*Antibacterial coating may cause color spots. We also verified as to whether these color spots affect antibacterial performance or not.

Dilution rate / Base material	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	Result
《Negative Control》 Bacteria culture dish								Viable bacteria 3.3×10^{9}cells/mL Relative value 1
	Unmeasurable	Unmeasurable	Unmeasurable	Unmeasurable	Many	326	38	
《For comparison purposes》 Normal (stainless) shelf plate								Viable bacteria 1.9×10^{9}cells/mL Relative value 0.59
	Unmeasurable	Unmeasurable	Unmeasurable	Unmeasurable	Many	194	17	
《Subject of verification》 Shelf plate with antibacterial coating								Viable bacteria Less than 100cells/mL Relative value Less than 3.1×10^{-8}
	Less than one for convenience sake	0	0	0	0	0	0	
《Subject of verification》 Shelf plate with antibacterial coating (Color spots)								Viable bacteria Less than 100cells/mL Relative value Less than 3.1×10^{-8}
	Less than one for convenience sake	0	0	0	0	0	0	

[Results]

From the number of colonies in the 10-6 dilution sample, the viable bacteria count 24 hours after the fungus liquid had been applied was calculated backwards. It should be noted that in the sample cultivated on a shelf plate with antibacterial coating, no colony appeared, so it was treated as less than one. It can be considered that with the antibacterial coating, multiplication could be reduced to less than 1/19,000,000 in 24 hours compared to a normal stainless shelf plate in a condition where attachments of droplets containing coliform bacteria is assumed. Antibacterial coating can thus be said to have sufficient antibacterial effects. No differences in antibacterial effects were found on the parts with color spots.

MG-71C: Normal culture of cells — Checking if the antibacterial coating adversely affects a cell culture

Cell: HeLa (adherent cells); **culture medium:** EMEM with 10% FBS and antibiotics; **container:** φ90 mm dish for cell culture; **culture volume:** 10 mL
Cell density at the start: 9.3 x 103 cells/cm^2; **Culture condition:** 5% CO_2, +37°C ; **culture time:** about 69 hours; **Evaluation item:** cell density
Judgment criterion: equivalent to a culture using a normal shelf plate as positive control
Results: There were no problems. No significant differences were found when compared to results of a culture with a normal shelf plate.

	Static placement location of a dish	Cell density at the start	Cell density at the end	Average cell density	Average multiplication rate
《Positive control》 Normal shelf plate (Stainless)	A (top shelf)	9.3×10^3cells/cm^2	6.4×10^4cells/cm^2	6.7×10^4cells/cm^2	7.2 times
	B (middle shelf)		5.3×10^4cells/cm^2		
	C (bottom shelf)		8.2×10^4cells/cm^2		
《Subject of verification》 Shelf plate with antibacterial coating	A (top shelf)		7.8×10^4cells/cm^2	7.0×10^4cells/cm^2	7.6 times
	B (middle shelf)		7.6×10^4cells/cm^2		
	C (bottom shelf)		5.7×10^4cells/cm^2		

MG-71M: low-oxygen culture of cells — Apoptosis induction with low oxygen

Cell: PC12 (cell line derived from a pheochromocytoma of rat adrenal medulla, adhesive cell)
For normal culture: Culture medium: RPMI1640 with 10% FBS, 5% horse serum and antibiotics; Culture condition: 5% CO2, +37° C
For apoptosis induction: Culture medium: RPMI1640 with 2% FBS and antibiotics; Culture condition:1.0% O2 (to be adjusted by N2 gas); 5% CO2, +37° C; container: :φ35 mm dish for cell culture; Evaluation item: Apoptosis/Necrosis induction efficiency values (compared to those of literature data1) value)

[**Procedure**]

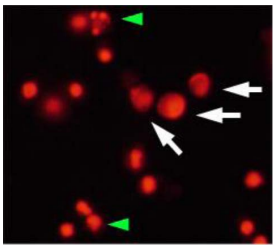
1) Cells are spread to be 5 x 104 cells/cm2 and cultivated for 24 hours (normal culture conditions).
2) Culture mediums replaced with one for an apoptosis induction. Cells were then divided into nine dishes and placed statically in positions as per the drawing below in the incubator and cultivated for 24 hours (normal culture conditions).
3) Cells were put into a low oxygen state and cultivated for a further 60 hours (condition for apoptosis induction).
4) Cells were collected and then double stained with PI and DAPI. By morphological observation, the ratio of cells that caused apoptosis and those that caused necrosis were counted.

[**Results**] While the number of apoptosis equivalent to that of literature data1) (average 81% vs. literature data 75%), no significant differences with static placement locations in the inside (minimum value ÷ average value = 75% ÷ 81% = 0.93, with a pass/fail criteria of 0.75.).

▼Static placement location of a dish

【Top shelf】			【Middle shelf】			【Bottom shelf】		
1		2				5		6
				9				
3		4				7		8

▶Morphological change after apoptosis induction



Green arrow: Apoptosis (nuclei are fragmented or condensed small), White arrow: Necrosis (the sizes and shapes of nuclei are similar to living cells)

Static placement location	Apoptosis	Necrosis	÷average value
1	83	17	1.02
2	81	19	1.00
3	79	21	0.98
4	80	20	0.99
5	79	21	0.98
6	86	14	1.06
7	82	18	1.01
8	75	25	**0.93**
9	84	16	1.04
Average	**81**	19	
In the case of 5% O2 (control)	3	0	
Literature data	75	25	
Pass/Fail criteria			0.75

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Reference

1)Yoshimura et al., Ceramide Formation Leads to Caspase-3 Activation during Hypoxic PC12 Cell Death. *J. Biol. Chem.* (1998) **273**, 6921-7.

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