Cell culture FAQs

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1. Observation after thawed cells:

1-1 Microscopic observation	Causes	Solutions
1-1-1 When the numbers of viable cells are not enough	1. Centrifuge to remove DMSO residue after the cells were thawed, some cells will not precipitate efficiently. As result, the number of cells will decrease. 2. Some cells are more likely to condense on the bottom of the vial. If not properly pipette, only the upper cells can be collected.	1. Dilute cells in pre-warm culture medium and seed in culture dishes or flasks. Change medium to remove DMSO after few hours or overnight after cells completely attached on the bottom of culture dish. 2. Slowly pipette thawed cells 5 to 7 times thoroughly.
1-1-2 The low viability	**1-1-2-1** Some cells have low viability or less cell number. **1-1-2-2** DMSO are toxic after the cells was thawed: 1. Some cells are more sensitive to the toxic of DMSO. e.g. HL-60 2. Cells are stay in the thawed vial too long. 3. The thawed cells are seeded in not enough culture medium to dilute the DMSO.	Resuscitation of frozen cell line in the T25 flask first. 1. The thawed cells must to spin down to remove the DMSO. 2. Have everything ready before thaw the cells. 3. Add at least 10ml fresh medium or make the final concentration of DMSO is less than 1%.

	1-1-2-3 Damage costs by temperature changes: 1. Do not thaw rapidly and culture or store in -80℃ refrigerate too long after receiving the cells. 2. Do not Transfer vials in a sealed container with dry ice or liquid nitrogen. 3. Allow to thaw until a small amount of ice remains in the vial, and then transfer the ice into fresh medium.(did not thaw cells completely) 4. Some cells need to culture in 28℃, so is the medium. If using 37℃, it may cause the low viability of the cells.	1. The best way is to thaw rapidly and culture or stores in -80℃ refrigerate no more than 2 days, also transfers into liquid nitrogen tank the day after receive. 2. Transfer vials in a sealed container with dry ice or liquid nitrogen. 3. Immediately thaw the cells completely. Wipe the outside of the tube with 70% alcohol, then transfer to class II safety cabinet. 4. Pre-warmed medium to the appropriate temperature and remove vial from container and place in a waterbath at required temperature e.g. fish cells, insect cells, etc.
	1-1-2-4 **Osmotic damage** 1. The thawed Cells are diluted rapidly into pre-warm growth medium. 2. Using PBS to dilute out the toxic of DMSO after the cells was thawed.	1. Transfer the cells from the thawed vial to a flask and then add fresh medium slowly and dropwise, or with warm medium in the flask, slowly add suspension cells along the wall of the flask into the medium. 2. Using pre-warmed growth medium to dilute out the DMSO.

	1-1-2-5 Use the different medium other than we recommend.	Before thaw the cells, please always using the medium that recommended. You can change the medium when cells are in log phase and freeze some cells for backup. Then using the remaining cells to change medium gradually. .

2. Observation after culture and sub culture**:** (references 1 , 2)

2-1 Macroscopic observation according to the color of the medium	**causes**	**solutions**
2-1-1 yellow and turbidity	Possibly bacteria or yeast contamination.	discard the culture
2-1-2 yellow and clear	1. Cells become confluent or over confluent. 2. The pH of the medium is acidic, not cause by the cell metabolism but cause by the high CO2 level in the incubator. 3. Mycoplasma contamination.	1. Subculture immediately. 2. Set CO2 at a required level for each cell and check for the supply of CO2 frequently. 3. Mycoplasma testing. Discard the contaminated culture.
2-1-3 bright purple red	The pH of the medium is alkaline 1. The most possible reason will be the low concentration of CO2 in the incubator. 2. The cap is too tight.	1. Check for the supply of CO2. 2. Loosen the cap.
2-1-4 cloudy floats	Fungal contamination. This kind of contamination will not make the medium turn yellow; however, hyphae will detected by microscopic observation.	Discard the contaminated culture. Also inspect the incubator to make sure no contaminated medium is spill out to induce the growth of the fungal.
2-2 Microscopic observation	**causes**	**solutions**
2-2-1 The poor growth condition of cells (see note[a])	1. Mycoplasma contamination 2. The low degree contamination cause by bacteria. 3. Alter composition of medium. Some cells are sensitive to this kind of	1. Mycoplasma testing. Discard the contaminated culture. 2. Microbial testing. 3. Pay extra attention to additives to the medium.

	changes. e.g. change serum, out of date glutamine and other additives, poor water quality or without adding some additives such as nonessential amino acid. 4. Over-trypsinzed the adherent cells or spilt ratio is too high.	4. When trypsinized, examine the cells using an inverted microscope to ensure that all the cells are detached. Trypsin should be neutralized with serum. Beware of the expiration date of the trypsin avoid to loss the activation of the trypsin.
2-2-2 A string of connected budding cells	Yeast forms a string of connected budding cells. Yeast size is 5 to 10 times smaller than the cells.	discard the culture
2-2-3 Reduced growth rate	Usually caused by mycoplasma, however, there are some other reasons: 1. Media are overdue or do not properly stored. 2. Glutamine is out of date. 3. Serum--change to different lot or brand or using the wrong kind of serum, plus the concentration of the serum is not right. 4. The temperature or CO2 concentration of the incubator is not right. 5. Should use the cell culture degree equipment and medium. 6. Perform the non-proper procedure to handle cells. For example: treating cells	

	with trypsin too long, cells become over-confluent to subculture, and the split ratio is too high.	
2-2-4 Alter cell function	1. Using the wrong medium (cells are still can grow in the different medium). 2. Keeping cell lines continually in culture as result in natural selection.	Should discard the culture, unless the change has research value.

note[a]: The poor growth condition of cells may look like:
Suspension cells will attach a little, dark, poor growth condition, rough surface, some bubbles around the cells and lots of cell debris. Besides characteristics mentioned above, adherent cells may also loosely attach to the flask.

References:

1. Darling, D.C. and Morgan, S.J. (1994) Animal cells culture and media. Wiley. John Wiley& Sons. Inc., publication.
2. Freshney, R.I.(2000) Culture of animal cells.4th ed. Wiley-Liss. John Wiley& Sons. Inc., publication.