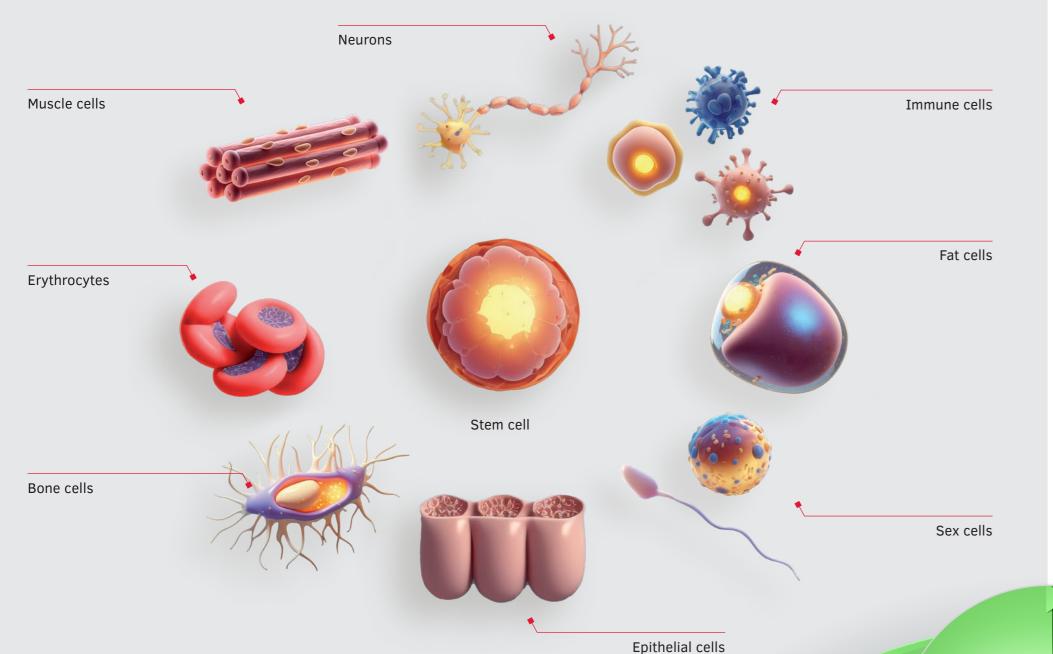
Because your cells are more than just lab residents!





1. Cell isolation

In order to understand cellular processes and behaviour inside tissues, breaking the tissue apart and singularizing the cells an organism one needs to understand the individual cells and by using cell strainers is essential before they can be introduhow they interact. Therefore tissues or liquid samples are ced into the *in vitro* culture vessel. extracted from laboratory animals or from patients. For solid



Cell isolation

(Animals/human patients)

2. Cell cultivation and analysis

For adherent cells that grow in a monolayer, the cell yield is Most cells need a minimal cell density for optimal growth. If proportional to the available surface of a culture vessel. Small the necessary initial cell count is not available, the cells must volumes and parallel experiments are best performed in mul- first be cultivated using a suitable vessel to increase the cell tiwell plates, which have a specific number of small wells. Larger quantity. dishes or flasks should be used if more cells are required

Cheat she	et to prepare	your culture	e vessel						
Vessel description	Catalog numbers	Surface area (cm²)		Recommended initial cell count at seeding*	Expected cell count at confluency*		Recommended working volume for dissociation (ml) e.g. with trypsin	Recommended working volume for cultivation (ml)**	Maximal working volume for cultivation (ml)
Flasks									
T-25	83.3910.XXX	24.7	Cells per flask	~ 7 x 10 ⁵	$\sim 3 \times 10^6$	Millilitres per flask	3	7	12.5***
T-75	83.3911.XXX	74.2		~ 2.1 x 10 ⁶	~ 1 x 10 ⁷		5	21	55***
T-175	83.3912.XXX	174.5		~ 5 x 10 ⁶	~ 2.2 x 10 ⁷		17	50	125***
Plates									
6-well	83.3920.XXX	9.07	Cells per well	~ 3 x 10 ⁵	~ 1.2 x 10 ⁶	Millilitres per well	1	4	15.75
12-well	83.3921.XXX	3.65		~ 1 x 10 ⁵	~ 5 x 10 ⁵		0.7	2	6.64
24-well	83.3922.XXX	1.82		~ 5 x 10 ⁴	~ 2 x 10 ⁵		0.25	1	3.39
48-well	83.3923.XXX	0.64		~ 2.5 x 10 ⁴	~ 8 x 10 ⁴		0.15	0.5	1.27
96-well	83.3924.XXX	0.29		~ 1 x 10 ⁴	~ 4 x 10 ⁴		0.08	0.2	0.39
Dishes									
35 mm	83.3900.XXX	9.4	_	~ 3 x 10 ⁵	~ 1.2 x 10 ⁶	Millilitres	1	3	8.3
60 mm	83.3901.XXX	22.1	Cells	~ 8 x 10 ⁵	~ 2.5 x 10 ⁶		3	5	26.1

*** Indicated by the dotted line in the graduation of SARSTEDT cell culture flasks.

cell nucleus

endoplasmatic

reticulum

vesicle



Maintenance & amplification of cells

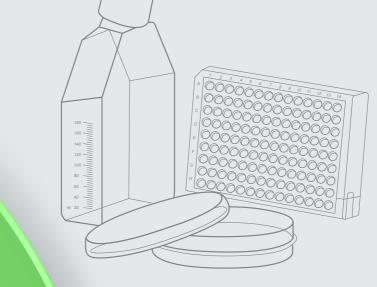
Cell cultivation

cell membrane

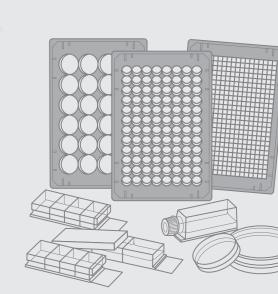
(New) start of cell cultivation (seeding Golgi apparatus

mitochondrion

cell plasma

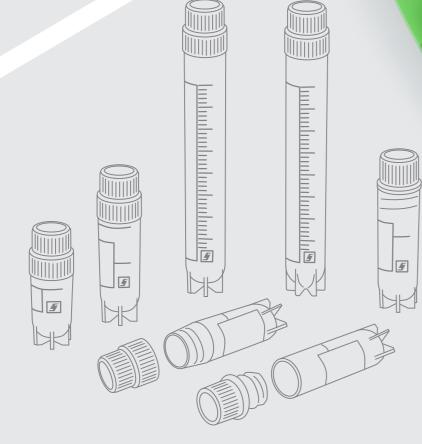


Experiment/analysis



96.7

244.0





For vital long-term cell conservation, cells are frozen in specialized freezing medium and stored in cryotubes at down to -196°C. The concentration of the cell suspension increased pressure to be frozen is typically between 10⁶ and 10⁷ cells per ml.

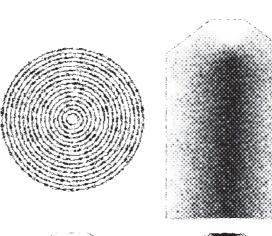
- We care about your safety, therefore please note:
- Pay attention to the marking of the nominal volume and only fill the tube to this point - Volume below cause an increased contamination risk due to possible penetration of liquid nitrogen upon improper storage
- Volume above could lead to exploding tubes during thawing due to
- Make sure to only close the tubes by hand to not overtighten it
- Make sure the cell suspension slowly freezes from the bottom up to prevent pressure buildup in the tube and retain cell viability
- Store the CryoPure tubes only in the gas phase of liquid nitrogen

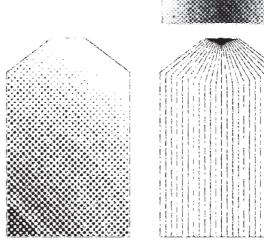
Freezing medium composition

70-75% (v/v)	Culture medium				
15-20% (v/v)	Serum (protective function > higher concentration for sensitive cells)				
10% (v/v)	DMSO or glycerol				

Conditions influencing cell culture







Troubleshooting

Patterned growth / No growth in some areas

- If vibrations occur within the culture vessel before the cells have attached, they will organize into a ring-like pattern. It is therefore key to eliminate the sources of the vibrations which can originate from the most unlikely sources like an old fridge nearby, a construction site or heavy doors closing forcefully on repeat.
- If you encounter no cell growth in the middle of your culture vessel, it is possible that you used too little growth medium. If the meniskus is too low in the middle of the vessel, cells will only grow on the edges of the vessel. Please always stick to the recommended working volume (see table) and check for evaporation regularly.
- If cells only grow on one side of the vessel, please check whether the incubator (shelf) is level. ■ The surface of cell culture vessels undergo treatment to facilitate cell attachment. The
- treated surface is sensitive to mechanical stress. If for example the surface is scratched with serological pipettes during cell seeding, lines without cell growth will be visible.