# Because your cells are more than just lab residents!

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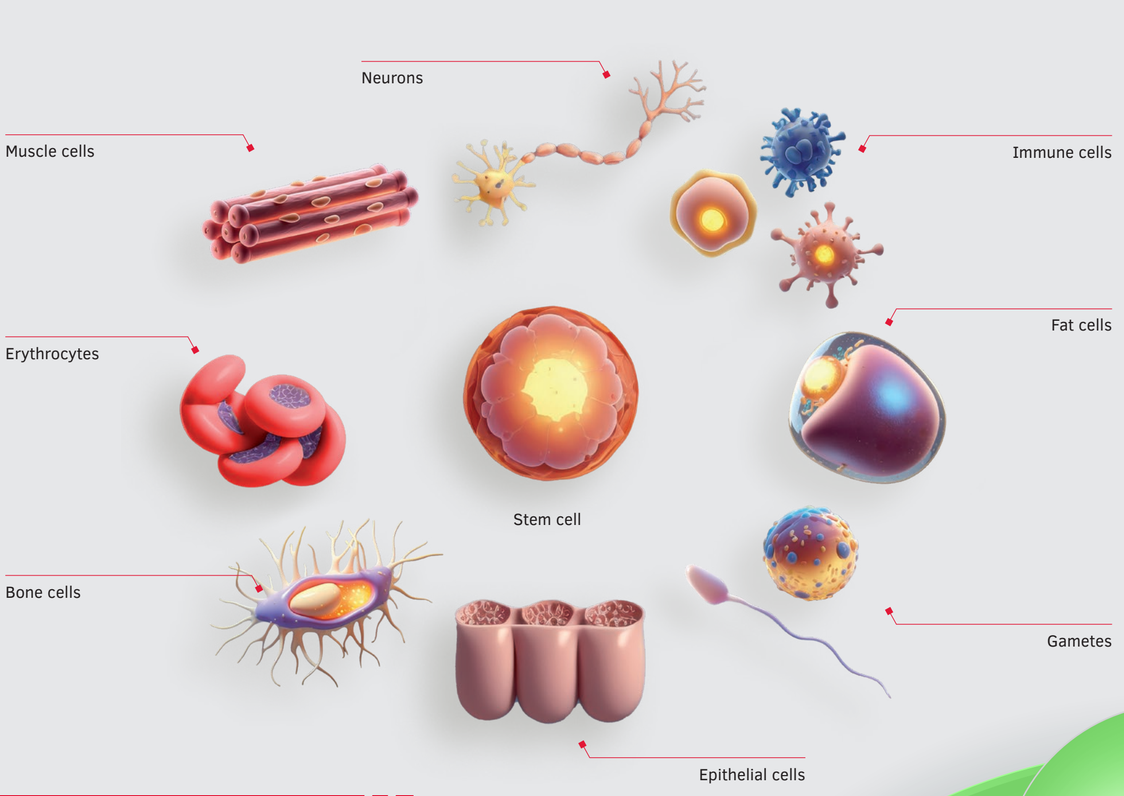
## 1. Cell isolation

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

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## 2. Cell cultivation and analysis

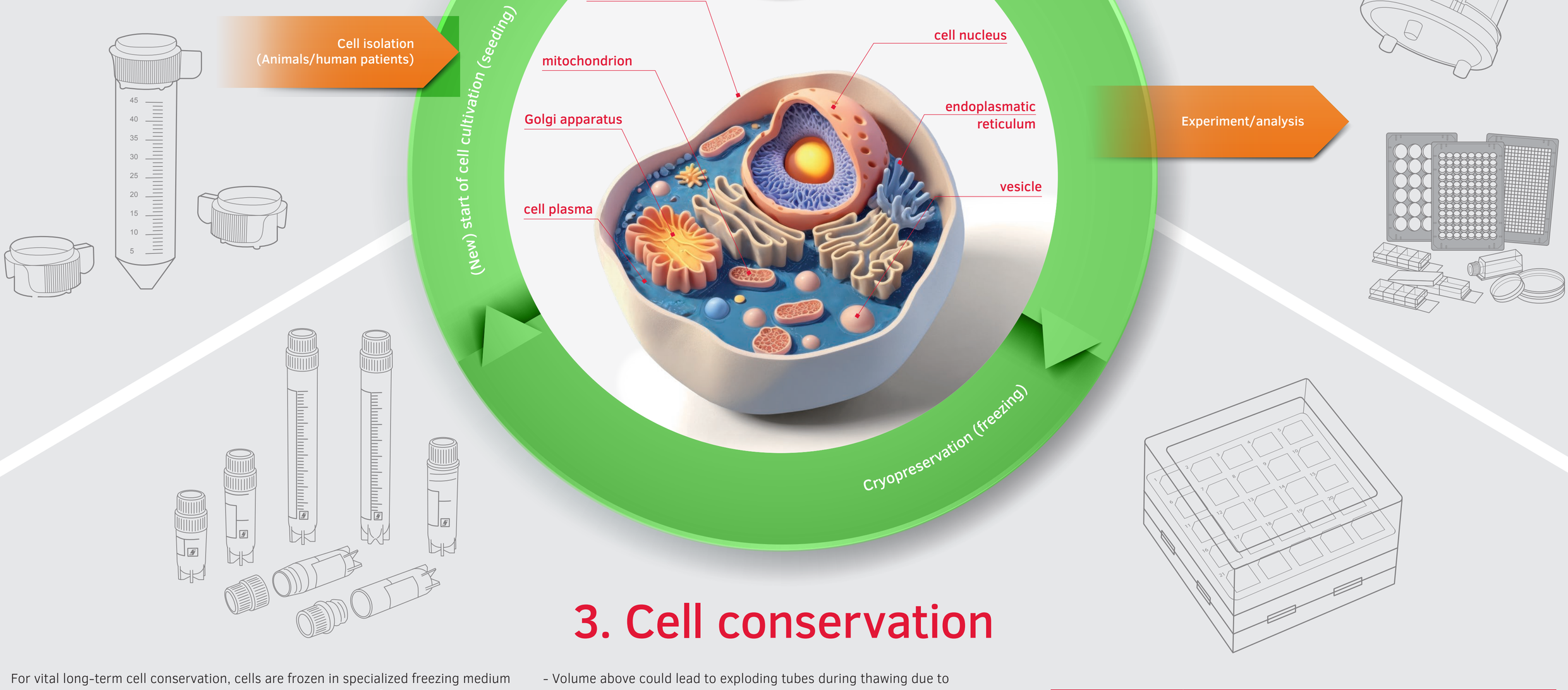
For adherent cells that grow in a monolayer, the cell yield is proportional to the available surface of a culture vessel. Small volumes and parallel experiments are best performed in multiwell plates, which have a specific number of small wells. Larger dishes or flasks should be used if more cells are required.

Most cells need a minimal cell density for optimal growth. If the necessary initial cell count is not available, the cells must first be cultivated using a suitable vessel to increase the cell quantity.

### Cheat sheet to prepare your culture 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 | ~ $7 \times 10^5$ | ~ $3 \times 10^6$ | Milliliters per flask | 3 | 7 | 12.5*** |
| T-75 | 83.3911.XXX | 74.2 | | ~ $2.1 \times 10^6$ | ~ $1 \times 10^7$ | | 5 | 21 | 55*** |
| T-175 | 83.3912.XXX | 174.5 | | ~ $5 \times 10^6$ | ~ $2.2 \times 10^7$ | | 17 | 50 | 125*** |
| **Plates** | | | | | | | | | |
| 6-well | 83.3920.XXX | 9.07 | Cells per well | ~ $3 \times 10^5$ | ~ $1.2 \times 10^6$ | Milliliters per well | 1 | 4 | 15.75 |
| 12-well | 83.3921.XXX | 3.65 | | ~ $1 \times 10^5$ | ~ $5 \times 10^5$ | | 0.7 | 2 | 6.64 |
| 24-well | 83.3922.XXX | 1.82 | | ~ $5 \times 10^4$ | ~ $2 \times 10^5$ | | 0.25 | 1 | 3.39 |
| 48-well | 83.3923.XXX | 0.64 | | ~ $2.5 \times 10^4$ | ~ $8 \times 10^4$ | | 0.15 | 0.5 | 1.27 |
| 96-well | 83.3924.XXX | 0.29 | | ~ $1 \times 10^4$ | ~ $4 \times 10^4$ | | 0.08 | 0.2 | 0.39 |
| **Dishes** | | | | | | | | | |
| 35 mm | 83.3900.XXX | 9.4 | Cells per dish | ~ $3 \times 10^5$ | ~ $1.2 \times 10^6$ | Milliliters per dish | 1 | 3 | 8.3 |
| 60 mm | 83.3901.XXX | 22.1 | | ~ $8 \times 10^5$ | ~ $2.5 \times 10^6$ | | 3 | 5 | 26.1 |
| 100 mm | 83.3902.XXX | 58.8 | | ~ $2.2 \times 10^6$ | ~ $8.5 \times 10^6$ | | 5 | 13 | 96.7 |
| 150 mm | 83.3903.XXX | 151.4 | | ~ $5 \times 10^6$ | ~ $2 \times 10^7$ | | 10 | 36 | 244.0 |

* Might differ depending on the cell type and needs to be determined when establishing a new protocol in your lab by using different seeding densities in preliminary experiments.
** Liquid covers the entire surface. The depth of the medium is adequate for cultivating most cell types.
*** Indicated by the dotted line in the graduation of SARSTEDT cell culture flasks.




## 3. Cell conservation

For vital long-term cell conservation, cells are frozen in specialized freezing medium and stored in cryotubes down to -196°C. The concentration of the cell suspension to be frozen is typically between $10^6$ and $10^7$ cells per ml.

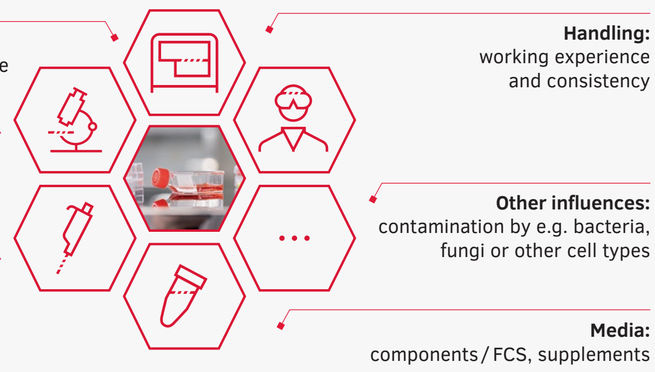
**We care about your safety, therefore please note:**

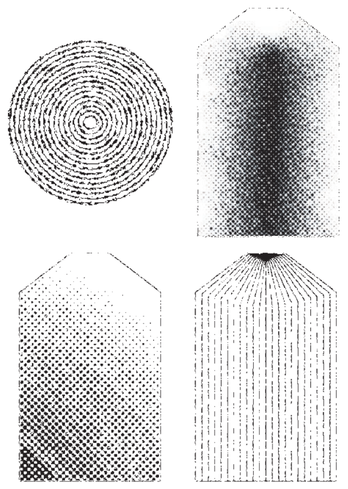
- Pay attention to the nominal volume marking and only fill the tube to this point
  - Volume below could cause an increased contamination risk due to possible penetration of liquid nitrogen upon improper storage
  - Volume above could lead to exploding tubes during thawing due to increased pressure
- Make sure to only close the tubes by hand and to not overtighten
- Make sure the cell suspension slowly freezes from the bottom up to prevent pressure buildup in the tube and to retain cell viability
- Store 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 / repeatedly.
- 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 meniscus 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.