provitro

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Passage kit 1

Cat.-No.: 204 0001

contains of:

255 0050	PBS
254 0025	Trypsin/EDTA solution
251 0025	Trypsin neutralization solution with FCS

Maintenance of Passage Kit

Immediately after delivery, place the passage kit in the dark at -20°C. Prior use thaw the passage kit. After thawing store the passage kit in the dark at 4°C for a maximum of 4 weeks. Take care: After thawing shelf time is limited to 4 weeks!

Subculture of normal human cells:

1. Examine the cell culture under the microscope. Proceed with subculturing if the cell culture reached the stage of confluence recommended for this particular cell type.
2. Incubate a freshly filled culture flasks in an incubator at 37°C, steam saturated with 5 % (V/V) CO_2. Make sure that the screw lids on the culture flasks are only slightly closed so that a gas exchange is possible.
3. Allow all three solutions in the passage kit to reach room temperature, and remove the seals from the bottles.
4. Open the cell culture flask carefully in a laminar flow cabinet and remove the medium using a sterile pipette. Do not touch the cell monolayer with the pipette. Replace the medium with PBS and wash the cell monolayer for about 30 seconds by gently swivelling the culture flask.
5. For standard cell culture flasks:

Tissue culture flask base	25 cm²	75 cm²
approx. media volume	5 ml	15 ml

6. Replace the PBS with approx. 80 µl of Trypsin-EDTA-solution/cm². Incubate the culture flask for 4 to 7 minutes at 37°C. The incubation period with the Trypsin-EDTA-solution should not exceed a total of 7 minutes.
7. For standard cell culture flasks:

Tissue culture flask base	25 cm²	75 cm²
approx. media volume	2 ml	6 ml

8. Immediately afterwards, examine the cells under the microscope. Make sure that all cells are detached. When the cells are completely detached, add approx. 80 µl of neutralising solution/cm² of culture flask surface. Transfer the cell suspension into a centrifuge tube. Rinse the culture flask with additional 80 µl of medium/cm² of culture flask surface and add this suspension to the one in the centrifuge tube.
9. For standard cell culture flasks:

Tissue culture flask base	25 cm²	75 cm²
approx. media volume	2 ml	6 ml

10. Centrifuge the suspension at 250 x g for 5 minutes. Make sure that the supernatant is completely clear and that all cells are packed in the sediment at the bottom of the tube. After that remove the supernatant carefully.
11. Add 2 ml of medium and re-suspend the cells slowly and carefully by means of a sterile pipette. Take a precise amount of the suspension to determine the cell number.
12. Dilute the cell suspension to a concentration required for culturing. Provitro recommends 200 µl medium/cm² of culture flask bottom.
13. For standard cell culture flasks:

Tissue culture flask base	25 cm²	75 cm²

approx. media volume 5 ml 15 ml

14. Remove the culture medium from the flasks prepared according to step 2 of these instructions. Transfer the cell suspension to these flasks.
15. Place the cell culture flasks newly seeded with subcultured cells in an incubator at 37°C, steam saturated with 5 % (V/V) CO_2. Close the screw lids on the culture flasks by half a turn to allow a gas exchange between flask and incubator.
16. Examine the cells microscopically after 24 hours. At least 80 % of the cells should adhere. Some cells will swim in the medium or only adhere slightly while most of the cells should be spread out on the bottom of the flasks. At this stage, most of the cells will grow alone or in small clusters. Once the cells have adhered (after min. 24 hours), remove the medium with a pipette and replace it with the same volume of fresh, pre-warmed medium.
17. Incubate for a further 24 hours. After this period, the culture should show mitotic clusters reflecting the proliferating activity of the cells.
18. Now, replace the medium only every two days.

Stability and storage:

After thawing the passage kit can be stored in the dark at 4°C to 8°C for up to 1 month. Do not heat the kit over 37°C or use uncontrollable sources of heat (e.g. microwave appliances).
Do not refreeze the kit. This can lead to high salt concentrations by freezing out pure water which will cause irreversible damage.

Special note:

Overtrypsination causes irreversible damage.
Because of the tryptic activity of this passage kit, do not exceed the recommended incubation period. Exposing the cells too long will cause irreversible damages to your culture.

Quality control:

Provitro's passage kit is thoroughly tested after each production. All components are tested in a stringent biological assay.