Product Protocol - PALM® DuplexDish

Cat. No. 1440-0550

Laser micromanipulation methods are used worldwide in the field of life science research. Up to now they were limited to fixed, non-vital biological specimen. Recently, new cell culture protocols and special equipment are developed that allow microdissection and separation of living cells with subsequent recultivation (Mayer et al. 2002). It has also been demonstrated that **L**aser **M**icrodissection and **P**ressure **C**atapulting (LMPC) has no influence on the viability or the proliferation rate of isolated cells. Even frequently re-cultivated cell colonies are still viable after several LMPC-isolation procedures (Stich et al. 2003). The PALM® DuplexDish is a culture dish especially adapted for LMPC. Adherent growing cells can be seeded in these double membrane dishes for subsequent selective isolation by LMPC.

1. Materials needed for LMPC of living cells

- Duplex Dish Cat. No. 1440-0550
- CapHolder for Life Cells for the CapMover, cat. no. 1210-0300
  or the LifeCell Collector, cat. no. 1210-0310
  used in conjunction with PALM® LPC-Microcentrifuge Tubes, cat. no. 1440-0200
- Appropriate cell culture medium including all necessary supplements

Please prepare all necessary material for recultivation prior starting with microdissection, as the cells will be covered with a minute amount of medium only while preparation; thus the collecting time is limited. *It may occur that the selected area can't be isolated with the catapulting impulse. To avoid this it is sufficient to initialize the dry DuplexDish by cutting and catapulting a small area (approx. 10000 µm^2) with the RoboLPC prior to cell culture.*

2. Cell culture and LMPC of living cells

(1) Adherent growing cells are seeded into the DuplexDish and are cultivated as usual in approx. 4 ml medium of choice up to the desired cell density. Usually after 1-2 days the cells are ready for microdissection, depending on the proliferation rate of the cell type.

(2) Prior to isolation of selected living cells the CapHolder for Life Cells has to be equipped with a sterile cap of a PALM® microfuge tube filled with 40 µl (max.) of medium used for later cultivation.

(3) For LMPC collection of cells the medium has to be removed from the DuplexDish, except for a residual amount of liquid. The prepared cap located in the CapHolder has to be centred directly above the selected area.

Note: The 20x and 40x objectives are most suitable for LMPC of living cells. Please check the correct setting of the LD-objective correction ring (due to the thin membrane, the setting should be set to almost zero!).

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(4) Now regions of interest from a single individual cell to larger tissue areas can be selected by any of the software marking tools. These areas are cut out (JointCut) together with the underlying LMPC-membrane. The cell-membrane stacks are subsequently catapulted by a single laser impulse at the border of the circumscribed area into an appropriate collection vial. Alternatively the RoboLPC function for automatically combined cutting and catapulting may be used.

Note: Laser settings of the MicroBeam need to be optimized prior to microdissection. Starting from the factory defaults, the optimal laser focus is likely one step higher. For catapulting, the difference between cutting and catapulting energy should be within 15-30 steps (Δ Energy), whereas the laser focus (Δ Focus) should differ -2 steps from cutting. With optimal settings even large areas (e.g., 380 x 250 µm) can be easily harvested. As a matter of principle, the cutting energy should be set as low as possible when working with living cells. Additionally, it is recommended to adjust the cutting speed at a lower level.

(5) After LMPC successful catapulting can be checked at the checkpoint position. The harvested area should now be located in the collection cap (best: 10x objective).

Note: If catapulting was not successful, energy and focus settings are to adjust. Induced by residual humidity, it may occur that the separated cell areas can't be catapulted with a single laser pulse. In this case, the difference between cutting energy and LPC energy should be increased (ΔFocus=20 to 25) for the first catapulting pulses.

3. Recultivation of isolated cells

(1) The cap containing the catapulted cells is put onto a sterile microfuge tube and spun down immediately at 250g for 5 minutes.

(2) The centrifuged cell-membrane stacks are now situated at the bottom of the microfuge tube. From there, the cells are transferred with a pipette into, e.g., a 24-well culture plate supplied with 1 ml of supplemented medium.

(3) Now the cells are grown under routinely used cell culture conditions. The harvested cells enter the cell cycle and start to proliferate usually within two days, depending on the cell type and the density of selected cells. However also the initially used PALM® DuplexDish, from which the isolation of the cells took place, can be put back into the incubator for further experiments.

Note: It is advisable to start the first recultivation experiments using 50 to 100 cells as an exercise to gain experience.

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Cat. No.: 1440-0550

Package Size: 10 DuplexDishes, individual sterile packs

Literature:

Mayer A, Stich M, Brocksch D, Schütze K and Lahr G:
Going in vivo with laser- microdissection.
Methods Enzymol, 2002, 356: 25-33

Schütze K, Burgemeister R, Clement-Sengewald A, Ehnle S, Friedemann G, Lahr G, Sägmüller B, Stich M and Thalhammer S
Non-contact live cell laser micromanipulation using PALM® MicroLaser Systems.
PALM, 2003, Scientific Edition No.11

Stich M, Thalhammer S, Burgemeister R, Friedemann G, Ehnle S, Lüthy C and Schütze K:
Live cell catapulting and recultivation.
Pathol Res Pract, 2003, 199: 405-409

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