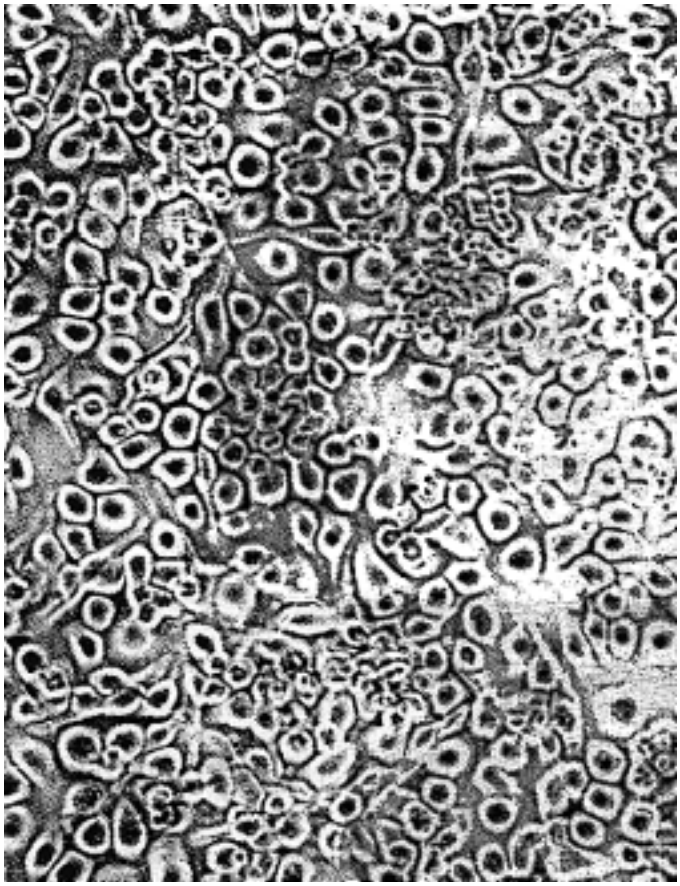
Tissue Culture



* **arium 611VF recommended for application**
Type I water purification system for low TOC and low endotoxin applications water quality to 18.2 MΩ × cm, endotoxin levels <0.001 EU/ml, TOC level < 1 ppb, product flow rate up to 1.5 l/min.

Preparation of primary cell cultures
For the preparation of single cells from tissues or organs in order to establish a primary cell culture, enzymatic dissociation has become the method of choice. Usually a combination of proteolytic enzymes is used, with enzyme preparations such as Trypsin, Collagenase, Dispase, Pronase, Elastase, Hyaluronidase being the most common products.

Trypsin and Pronase cause the most complete dissociation of tissue into single cells, but also damage cells more strongly. Dispase and Collagenase give lower yields of single cells, but do less damage to them during dissociation of the tissue. Other enzymes such as Hyaluronidase and Elastase are only used in combination with other enzymes. DNase is often added during tryptic dissociation of tissue in order to hydrolyze released DNA that would support aggregation of cells.

Enzymatic dissociation of tissues can be performed both at 37°C and at 4°C with time being a critical factor. Incubation at 37°C allows for more complete dissociation in a short time but will also result in more damage to the cells. On the other hand, incubation at 4°C can be safely carried out for extended time periods without much damage.

Generally, all tissues and organs should be obtained under aseptic conditions or using media containing twice the recommended concentration of antibiotics. All subsequent steps are performed in a laminar flow cabinet. All media used for washing and dissociation of tissues and all subsequently used culture media are prepared with **highly purified water***.

As a typical example, the preparation of heart muscle cells from chicken embryos is described as outlined in Cell and Tissue Culture (T. Lindl, ed.), Heidelberg | Berlin, 2000.

Chicken embryo hearts are washed with buffered saline and all blood vessels are carefully removed. The hearts are cut into small pieces using a scalpel and washed again with saline. The small pieces are transferred into dissociation medium with a sterile pasteur pipette.

Dissociation medium is prepared by dissolving 5 mg of Trypsin in 20 ml of buffered saline. Alternatively, Collagenase (Worthington Type II) adjusted to an activity of 150–200 U/ml, corresponding to 10–20 mg of Collagenase per 20 ml of buffered saline, can be used.

The flask with the tissue fragments and the enzyme solution is incubated at 37°C for 15 min. (Trypsin) or 1 hr (Collagenase) with gentle shaking on a rotary shaker | incubator such as CERTOMAT IS, B.Braun Biotech International.

The suspension is broken into single cells by carefully aspiring it several times into a sterile 10 ml pipette, and transferred into a sterile 50 ml centrifuge tube. The same volume (20 ml) of medium containing 10% fetal calf serum is added, and the sample is centrifuged for 10 min at approx. 200 × g.

The supernatant is removed, the cell pellet washed twice in serum-containing medium and finally resuspended in medium containing 15% horse serum. Cell concentration should be approx. 3×10^5 cells/ml as initially established by counting in a gridded chamber.

Cells are disseminated at a density of approx. 5×10^5 cells per 10 cm^2. Depending on the density of the cell suspension, the heart cells will resume pulsation after 24–48 hrs, whereas the fibroblasts remain immobile.

A relative enrichment of muscle cells can be obtained by pipetting the cell suspension into a large culture flask (150 cm^3), taking off the supernatant after 30 min and transferring it into culture dishes.

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W/sart-000 · G
Publication No.: SLG2012-e01041
Order No.: 85030-514-72

CERTOMAT IS, a true bench-top incubator-shaker, has the smallest footprint (540 × 680 mm) of all BBI environmental shakers. Available in 25 mm or 50 mm orbits, the CERTOMAT IS, is ideal for any shaking application requiring controlled speed (40 to 400 rpm) and temperature (RT +5°C to +60°C). The instrument can be used at ambient temperature of between 10°C to 60°C and is optionally equipped with refrigeration (RT –10°C to +60°C). A wide range of trays with clamps and hinged racks are available as accessories.