

System for suspension cell manipulation & Piezo assisted manual injection into Animal cells and/or Embryos.

System Description:

System consists of two Manipulators and two manual injectors, one manipulator for moving and positioning the injection needles in the field of view of the microscope for precisely manipulating, picking up and injecting cells and/or solutions (eg., DNA) into the egg cells / embryos (oocytes) using a hydraulic manual Injector to supply the intended pressure and vacuum. The second Manipulator is used to hold the egg cell / embryo (Oocyte) precisely in position using air vacuum produced with the aid of a pneumatic manual Injector while the cells / Nucleic acid (DNA/RNA) is being injected into it. The system should be flexible enough to be installed, adapted & used on left or right side of all commonly used microscopes (e.g., inverted microscopes, upright microscopes and stereo microscopes). The needles used for such precise injections would have to be of reproducible quality through narrowly defined specifications and intensive quality control, as well as the greatest security through effective sterilization methods. Specialized Pipette tip which can be used both for filling the microinjection capillaries with minute amounts of liquids.

A. Micromanipulator features:

The system should be a fully electronic system. All X, Y, Z axis movements are controlled electronically.

All three axis movements should be controlled with one single joystick

The system should have resolution of not more than 0.02 μm increment for fine and smooth movement.

The system should have a Dual Speed joystick: proportional/direct movement and dynamic movement. With the proportional movement, the movement of the joystick is directly transmitted to the movement of the capillary. With the dynamic movement, the speed of the capillary is increased by the degree of joystick deflection.

The system should have a tool travel speed of at least 10,000 $\mu\text{m}/\text{s}$ for rapid work and penetration of tough membranes such as zona pellucida.

The control panel should preferably have an LCD display that shows coordinates of the motors and the functions being used.

The system should have the coarse, fine, extra-fine movement selections using a single upright joystick.

The speed of the capillary should be adjustable using an ergonomic turn wheel on the control panel to fit user's skill and different injection steps.

The system should have a programmable Z axis limit as defined injection level in order to avoid capillary breakage during injection. This partial automation would particularly be preferred.

The system should have an automated function for the capillary to move out of working radius and return to the precise same spot to save time in changing capillary.

The system should be able to store up to 5 independent working positions in all three coordinates (X, Y, Z) precisely. These positions can be recalled anytime either via the softkeys on the control panel or the joystick button. Furthermore, the position storages should be remembered after switching off/on of the device.

The system should have at least 4 pre-defined programs for common applications and free definable soft keys to allow users to customize a program.

The system should be ergonomically designed where the user shall be able to rest his/her hand on the control board of the system for long period of handling to reduce strain of hands, arms and shoulders. The system should be able to be updated from time to time with newer software versions using the USB port, without the need to purchase new devices.

The system should be able to be connected to an electronic microinjector to allow movement of the capillary and injection to be carried out automatically.

The system should be able to connect to a piezo device and the piezo impulses can be supported by adjustable automated forward movements.

The system should be able to adapt to all common inverted- and stereo-microscopes.

B. Microinjector features

General features of the injector:

Maintenance-free piston/cylinder system Large, ergonomic dials Stable, non-slip base plate Universal capillary holder for mounting onto all common micromanipulators

Two microinjectors should be quoted with following features

1) Features of the Hydraulic Manual Injectors –For the injection side

Variable transmission ratio (1:1 and 1:10) for applications that demand high resolution and sensitivity such as the transfer of organelles

Quick valve system for simple, rapid filling Max. pressure: 20,000 hPa

The maximal volume that can be set should be between 900-1000ul.

The minimal volume that can be set should range from less than or equal to 2nl with fine and about 20 nl with coarse

2) Features of the Pneumatic Manual Injectors for holding side Resolution should be optimal for holding suspension cells Pressure-compensation valve for setting work point Easy to use and no oil filling necessary

The volume range that can be set should range from 90ul to equal to or greater than 2.5ml

Features of the Accessory on the injection to generate Piezo-impluses

The piezo element/actuator is located at the front of the capillary holder

directly adjacent to the microcapillary and should transmit the piezo impulses directly and without loss onto the attached microcapillary.

The control unit should have at least two channels to set the two independent regimes of Piezo-impluse Speed and Intensity.

Control unit should be Easily and freely programmable with the option to saving favorite sets of parameters Parameters for both channels should be displayed at the same time and should be set independently by clearly visible touch buttons on the control unit Device should be Intuitive to use -triggering of the impulses during operation of the peizo module for both the channels that should be preferably effected by both the rotary knobs (A & B) on the control unit and through an optimized foot pedal control. Device should have an Ultra-fast and powerful cleaning function for removing cell debris during injections The actuator should be easy to install, dismantle and re-install.

Piezo-driven drilling unit should be largely free of lateral oscillations to achieve high efficiency of enucleation and nucleus transfers. Should be compatible with the micromanipulator being quoted.

Should be provided with a power cable 230 V 50Hz. Injection capillaries/needle features: Precision capillaries for SCNT Glass microcapillaries with 1 mm outer diameter of upper end Sterilized by validated gamma irradiation and Non cytotoxic to cells. Microinjection capillary for reproducible injections Each capillary should have Precise tip length accuracy Injection needles should be 100%

individually tested

Features of TransferTips -

- Injection needle: 4-15 micrometer inner diameter, 20 degree tip angle.
- Microloader for recovering surplus solution from capillary.

Features of Tips - For holding suspension cells (e.g., oocytes or blastocysts)

15 μm inner diameter 100 μm outer diameter Upto 35° tip angle

Installation, successful demonstration and warranty for atleast three years should be provided

Easy accessibility of technical service support